06/727,105

Attorney's Docket No.: 16947-005001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No.: 4,650,787
Patentee: Schally et al.
Issue Date: March 17, 1987
Serial No.: 727,150
Filed: April 25, 1985
Title : BIOLOGICALLY ACTIVE OCTAPEPTIDES

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL FOR APPLICATION FOR INTERIM EXTENSION OF PATENT TERM

Enclosed is a Application for Interim Extension of Patent Term (9 pages) and nine (9) exhibits. Exhibit 1 - 1 page of cover and13 pages of exhibit; Exhibit 2 - 1 page of cover and 2 pages of exhibit; Exhibit 3 - 1 page of cover and 3 pages of exhibit; Exhibit 4 - 1 page of cover and 1 pages of exhibit; Exhibit 5 - 1 page of cover and 1 pages of exhibit; Exhibit 6 - 1 page of cover and 1 pages of exhibit; Exhibit 7 - 1 page of cover and 3 pages of exhibit; Exhibit 8 - 1 page of cover and 11 pages of exhibit; Exhibit 9 - 1 page of cover and 2 pages of exhibit.

Enclosed is a check in the amount of $420.00 for the filing fee. Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: April 7, 2005

Scott B. Markow
Reg. No. 46,899

Fish & Richardson P.C.
1425 K Street, N.W.
11th Floor
Washington, DC 20005-3500
Telephone: (202) 783-5070
Facsimile: (202) 783-2331

40275015.doc

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No. : 4,650,787 Attorney Docket No. 16947-005001
Patentee : Schally et al.
Issue Date : March 17, 1987
Serial No. : 727,150
Filed : April 25, 1985
Title : BIOLOGICALLY ACTIVE OCTAPEPTIDES

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR INTERIM EXTENSION OF PATENT TERM UNDER 35 U.S.C § 156(d)(5)

I. Pursuant to 35 U.S.C. § 156(d)(5), H3 Pharma, Inc. ("H3 Pharma"), a corporation incorporated under the laws of Quebec, Canada, with its registered office at 666 Sherbrooke Street West, Suite 1400, Montreal, Quebec H3A 1E7, Canada, hereby applies for a one-year interim extension of patent term for United States Patent No. 4,650,787 ("the '787 patent," attached as Exhibit 1), The '787 patent covers Sanvar™ Injection ("Sanvar") and methods of using Sanvar.[1]

II. H3 Pharma certifies that it is the exclusive agent Debiopharm S.A. and Debio Recherche Pharmaceutique S.A. (collectively "Debio") for the purposes of pursuing a patent term extension for the '787 patent, by virtue of the Authorization for H3 Pharma to Apply for Extension of Patent Term ("Authorization," attached as Exhibit 2). In the Authorization, Debio certifies that Debio is the exclusive licensee of the '787 patent, by virtue of a license received from The Administrators of The Tulane Educational Fund ("Tulane"), the assignee of 100% of the right, title and interest in the '787 patent (see Exhibit 3), and that Tulane has exclusively granted all patent rights in the '787 patent to Debio, including the right to pursue patent term extensions. Debio has granted H3 Pharma a sublicense to the '787 patent, including the rights to develop and commercialize vapreotide.

III. Pursuant to 37 C.F.R. § 1.790, H3 Pharma provides the following information as required by 37 C.F.R. §§ 1.740 and 1.741:

[1] Sanvar is also known by the trade name Octastatin™.

1. The complete identification of the product currently undergoing regulatory review is Sanvar™ Injection. The active ingredient of Sanvar is vapreotide acetate, an acetate salt of vapreotide, which is a synthetic analogue of somatostatin. Vapreotide is an octapeptide having the following chemical formula:

D-Phe – Cys – Tyr – D-Trp – Lys – Val – Cys – Trp-NH_2

The line between the Cys residues indicates a disulfide linkage. The structural formula of vapreotide is shown in Exhibit 4.

Sanvar is manufactured according to the description in Drug Master File No. 17133 submitted to the Food and Drug Administration by Genzyme Pharmaceuticals on December 31, 2003. Briefly, vapreotide is synthesized by coupling peptide fragments in the sequence indicated above, using optically pure peptides and standard segment condensation protocols, in which α-amino and α-carboxyl groups and lateral side chains are protected as necessary. The octapeptide is formed by azide coupling of the two tetrapeptide fragments (synthesized in solution). The disulphide bridge is formed by iodine oxidation. The protected octapeptide disulphide is purified by chromatography and then deprotected by treatment with formic acid. The product is purified by reverse phase HPLC chromatography and vapreotide acetate formed by ion exchange of vapreotide TFA. The steps for synthesis of vapreotide acetate are outlined in Exhibit 5.

Sanvar is provided in single dose vials containing a sterile, lyophilized powder containing 0.6 mg vapreotide acetate (peptide base units) to be reconstituted to 50 mL with 0.9% Sodium Chloride Injection, USP, prior to infusion. The 0.9% Sodium Chloride Injection, USP, is not provided with Sanvar and does constitute part of the new drug application that is the basis for this application. Each vial of Sanvar contains a 10% overfill of vapreotide acetate to allow for losses during extraction and administration.

Sanvar reproduces most of the effects induced by natural somatostatin. In the pituitary gland, Sanvar inhibits the secretion of growth hormone. In the gastrointestinal tract, Sanvar inhibits the secretion of many endocrine digestive peptides, e.g., insulin, glucagon, gastrin, vasoactive intestinal peptide, secretin, and cholecystokinin. Sanvar also suppresses exocrine secretion from the pancreas and

gastrointestinal tract. In the new drug application that is the basis for this application, Sanvar is indicated for the treatment of esophageal variceal hemorrhage in patients with portal hypertension.

2. Under section 505(b) of the Federal Food, Drug and Cosmetic Act, regulatory review is ongoing of new drug application (NDA) number 21-761 for Sanvar, which was initially submitted on February 27, 2004.

3. Applicant reasonably expects that the regulatory review period for the new drug application will extend beyond the expiration of the term of the '787 patent. An approvable letter for Sanvar was mailed on December 21, 2004 (Exhibit 7).

4. Vapreotide acetate, the active ingredient in Sanvar, has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act.

5. Pursuant to 35 U.S.C § 156(d)(5) and 37 C.F.R. § 1.790(a), this application is being timely submitted during the period beginning six months and ending fifteen days before the term of the '787 patent is due to expire. The last day on which this application could be submitted is April 10, 2005.

6. The U.S. Patent for which an extension is being sought is United States Patent No. 4,650,787. The names of the inventors are Andrew V. Schally and Ren Z. Cai. The '787 patent was filed on April 25, 1985, issued on March 17, 1987, and will expire normally on April 25, 2005.

7. A copy of the '787 patent is attached hereto in Exhibit 1.

8. A copy of a Maintenance Fee Statement for the '787 patent, dated February 12, 2004 is attached as Exhibit 6. The Maintenance Fee Statement shows that the maintenance fees for the fourth, eighth, and twelfth years were paid on September 10, 1990, June 17, 1994, and August 17, 1998, respectively. Applicant certifies that no disclaimer, certificate of correction, or re-examination certificate related to the '787 patent has been issued.

9. As described below, claims 1, 3, 4, 6, and 14 of the '787 patent read on the product Sanvar.

Claims of '787 Patent	**How Claim Reads on Sanvar**
1. A compound of the formula A – C'' – X – Z – Lys – Y – C' – B wherein A is an L, D or DL amino acid selected from the group consisting of phenylalanine (Phe), its acetylated derivatives or a pharmaceutically acceptable acid addition salt thereof; B is an L, D or DL amino acid selected from the group consisting of threonine amid (Thr NH_2), tyrosine amide (Tyr NH_2), tryptophan amide (Trp NH_2); X is L-phenylalanine (L-Phe) or L-tyrosine (L-Tyr); Y is L-valine (L-Val); Z is D-tryptophan (D-Trp); and C'' and C' are L or D-cysteine (Cys), -aminobutyric acid (Abu), aspartic acid (Asp) or lysine (Lys); provided that where C' is Cys, C'' is also Cys and where C' or C'' are other than Cys, C'' is different from C' and is other than Cys; the connecting line between C'' and C' signifies a bridge selected from the group consisting of carbon/carbon, carbon/sulfur, sulfur/sulfur and amide bridges; and the pharmaceutically acceptable acid addition salts thereof.	Vapreotide, the active ingredient of Sanvar, is a compound of the formula: D-Phe – Cys – Tyr – D-Trp – Lys – Val – Cys – Trp-NH_2 wherein the line connecting the two Cys residues indicates a sulfur/sulfur bridge.
3. A compound according to claim 1 wherein C'' is Cys; X is Tyr; Z is D-Trp; Y is Val; and C' is Cys.	Claim 3 reads on vapreotide, as described above with respect to claim 1.
4. A compound according to claim 1 wherein C'' is Cys; X is Tyr; Y is Val; and C' is Cys.	Claim 4 reads on vapreotide, as described above with respect to claim 1.
6. A compound according to claim 1 which is – D-Phe – Cys – Tyr – D-Trp – Lys – Val – Cys – Trp-NH_2	Claim 6 reads on vapreotide, as described above with respect to claim 1.

Claims of '787 Patent	How Claim Reads on Sanvar
14. A pharmaceutical composition effective for reducing growth hormone serum levels which comprises an octapeptide of claim 1, its reduced form or a pharmaceutically acceptable acid addition salt thereof in a pharmaceutically acceptable liquid or solid carrier thereof.	Sanvar is effective for reducing growth hormone levels. Sanvar contains a sterile, lyophilized powder containing 0.6 mg vapreotide acetate, which is an octapeptide covered by claim 1, as described above. The powder is contained in a single dose vial to be reconstituted with 0.9% sodium chloride injection, USP, a pharmaceutically acceptable liquid carrier.

As described below, claim 16 of the '787 patent reads on a method of using Sanvar for the treatment of esophageal variceal hemorrhage in patients with portal hypertension, which is the indication set forth in the NDA.

Claims of '787 Patent	How Claim Reads on Method of Using Sanvar
16. A method of treating excess release of growth hormone, gastrointestinal disorders, and diabetes in a mammal in need of such therapy which comprises administering to said mammal an effective dose of octapeptide of claim 1, its reduced form, or a pharmaceutically acceptable acid addition salt thereof.	Sanvar is indicated for the treatment of esophageal variceal hemorrhage in patients with portal hypertension, which is a gastrointestinal disorder.

10. The relevant dates and information required pursuant to 35 U.S.C. § 156(g) in order to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

Investigational new drug (IND) application number 59,287, which covers Phase III testing of vapreotide acetate (i.e., Sanvar) for the treatment of bleeding due to pancreatic resection, was received by FDA on November 23, 1999. FDA acknowledged receipt of this application on December 1, 1999. FDA completed a Phase III protocol review for a study on bleeding during pancreatic resection on April 11, 2000.

New drug application (NDA) application number 21-761 for Sanvar, for the treatment of variceal hemorrhage due to portal hypertension associated with therapeutic endoscopy, was initially submitted to the Food and Drug Administration (FDA) on February 27, 2004.[2] A copy of the approvability letter for Sanvar is attached as Exhibit 7.

[2] While not relevant for obtaining an interim patent term extension, Applicant notes that, despite the different indications in the IND and the NDA, both would be used to calculate the amount of patent term extension of the '787 patent after the NDA is granted. Under 35 U.S.C. § 156(g)(1), the relevant time periods are IND testing and NDA review of a "drug product." For purposes of patent term extension, a "drug product" is defined as the "active ingredient" of the drug. 35 U.S.C. § 156(f). Because the IND and NDA both relate to the active ingredient vapreotide acetate, both would be relevant to calculating the amount of post-NDA patent term extension. In addition, much of the testing data from the IND has been used as part of the NDA, making the IND relevant to the calculation of post-NDA patent term extension. Moreover, in determining the length of regulatory review periods, the FDA has recognized that "The investigational path of a new drug is rarely straightforward. From the time of the first submission of an IND to the time, usually years later, of final approval for marketing, the course of drug investigation goes up many blind alleys and frequently takes off in new directions. Rarely, if ever, is a drug approved under precisely the same conditions (i.e., indication(s), patient population(s), dosing regimen(s), duration of treatment, use in conjunction with other drugs, etc.) for which it is initially investigated." 67 Fed. Reg. 65,358, 65,359 (Oct. 24, 2002).

11. Attached as Exhibit 8 is a brief description of the significant activities undertaken by H3 Pharma during the applicable regulatory review period with respect to Sanvar and the significant dates applicable to such activities. J. Kay Noel and Associates refers to H3 Pharma's regulatory agent in the United States.

12. The length of the interim extension requested is one year, pursuant to 35 U.S.C. § 156(d)(5)(B). In the opinion of H3 Pharma, the '787 patent is eligible for the interim extension herein applied for because it satisfies all of the requirements for such interim extension as follows:

35 U.S.C. § 156(a)

The '787 patent claims the product Sanvar and a method of using Sanvar.

35 U.S.C. § 156(a)(1)

The term of the '787 patent has not expired before submission of this application.

35 U.S.C. § 156(a)(2)

The term of the '787 patent has never been extended.

35 U.S.C. § 156(a)(3)

The application for interim extension is submitted by H3 Pharma, as the agent of Debio, which is the agent of Tulane, the owner of record, as discussed above.

35 U.S.C. § 156(a)(4)

The product, Sanvar, is subject to a regulatory review period before it can be commercially marketed or used.

35 U.S.C. § 156(a)(5)(A)

The commercial marketing of the product, Sanvar, after the regulatory review period indicated herein, will be the first permitted commercial marketing of the product under section 505(b) of the Federal Food, Drug and Cosmetic Act, under which such regulatory review is occurring.

13. H3 Pharma acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought by this application.

IV. By the power of attorney attached as Exhibit 9, H3 Pharma appoints Scott B. Markow, Reg. No. 46,899 and Anita L. Meiklejohn, Ph.D., Reg. No. 35,283, of Fish & Richardson P.C., as its attorneys to prosecute the application for patent term extension of U.S. Patent No.

4,650,787 and to transact all business in the Patent and Trademark Office connected therewith with full powers of substitution and revocation.

V. All inquiries and correspondence relating to this application for patent term extension should be directed to:

Scott B. Markow
Fish & Richardson P.C.
1425 K Street, N.W.
11th Floor
Washington, DC 20005
Telephone (202) 783-5070
Facsimile: (202) 783-2331

VII. The application is accompanied by two additional copies of the application.

VII. It is respectfully requested that this Application for An Interim Extension of Patent Term Under 35 U.S.C. § 156(d)(5) and 37 C.F.R. § 1.790 of United States Patent No. 4,650,787 be granted.

VIII. The prescribed fee of $420.00 for receiving and acting upon the application for extension is enclosed. Please apply all other charges or credits to Deposit Account 06-1050.

Respectfully submitted,

April 7, 2005
Date

Scott B. Markow
Reg. No. 46,899

Fish & Richardson PC
1425 K Street, N.W.
11th Floor
Washington, DC 20005
Telephone (202) 783-5070
Facsimile: (202) 783-2331
Enclosures: Exhibits 1-9

EXHIBIT 1

United States Patent Number 4,650,787

United States Patent [19]

Schally et al.

[11] **Patent Number: 4,650,787**

[45] **Date of Patent: Mar. 17, 1987**

[54] BIOLOGICALLY ACTIVE OCTAPEPTIDES

[76] Inventors: **Andrew V. Schally**, 5025 Kawanne Ave., Metairie, La. 70002; **Ren Z. Cai**, 2123 Perdido St., New Orleans, La. 70112

[21] Appl. No.: **727,105**

[22] Filed: **Apr. 25, 1985**

[51] **Int. Cl.**[4] **A61K 37/24**; C07K 7/26

[52] **U.S. Cl.** **514/11**; 530/311

[58] **Field of Search** 260/112.55; 514/11; 530/311

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,209,426	6/1980	Sarantakis	260/112.55
4,282,143	8/1981	Sarantakis	514/11
4,395,403	7/1983	Bauer et al.	514/11
4,435,385	3/1984	Bauer et al.	514/11
4,440,904	4/1984	Sarantakis	260/112.55
4,443,434	4/1984	Lion	514/11
4,451,394	5/1984	Sarantakis	260/112.55
4,496,543	1/1985	Bauer et al.	514/11

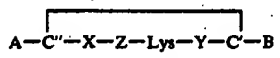
FOREIGN PATENT DOCUMENTS

0030920	2/1984	European Pat. Off. .
2125799	3/1984	United Kingdom .

Primary Examiner—Delbert R. Phillips
Attorney, Agent, or Firm—Omri M. Behr

[57] **ABSTRACT**

Novel compositions of the formula



wherein

A represents an L, D or DL amino-acid selected from the group consisting of Ala, Val, Phe, p-Cl-Phe, Trp, Pro, Ser, Thr, Glu, Gly, Beta Ala, Abu, N-Me Ala, 5-F-Trp, 5-Br-Trp, 5-Cl-Trp, their acetylated derivatives or a pharmaceutically acceptable acid addition salt thereof;

B represents an L, D or DL amino acid amide selected from the group consisting of Thr NH_2, Val NH_2, Pro NH_2, HO-Pro NH_2, Ser NH_2, Tyr NH_2, Trp NH_2, 5-F-Trp NH_2, For-Trp NH_2, Ala NH_2, Gly NH_2, Me Ala NH_2;

X represents L-Phe or L-Tyr,

Y represents L-Thr or L-Val;

Z is L, D or DL-5-F-Trp, 5-Br-Trp, 5-Cl-Trp, 5-I-Trp or D-Trp; and

C'' and C' represent L or D Cys, Abu, Asp or Lys; and the pharmaceutically acceptable acid addition salts thereof; are useful as agents for inhibiting the release of growth hormone, for the treatment of gastrointestinal disorders and for therapy of certain cancers and the management of diabetes. These biologically active octapeptides all possess a terminal amino acid amide at position 8 and are prepared by solid phase methods.

16 Claims, No Drawings

BIOLOGICALLY ACTIVE OCTAPEPTIDES

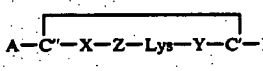
BACKGROUND OF THE INVENTION

Somatostatin is a cyclic tetradecapeptide which inhibits the secretion of pituitary growth hormone. Several analogs of somatostatin have been previously described. Verber et al., *Nature*, 292, 55, (1981) has reported the sythesis and the activity of a cyclic hexapeptide analog obtained by replacing 9 of the 14 aminoacids of somatostatin with a single proline. Additionally, the same group has reported hexapeptide derivatives of high potency, *Life Sciences*, 34, 1371 (1984). Bauer, et al.. in "Peptides" 1982, p. 583, Ed. K. Blaha, P. Malon—1983 by Walter De Gruyter & Co., Berlin, New York, describe the synthesis and activity of octapeptide analogs. (cf. also *Life Sciences*, 31, 1133, L982)).

In general, the abbreviations used herein for designating the amino acids and the protective groups are based on recommendations of the IUPAC-IUB Commission of Biochemical Nemenclature, see *Biochemistry*, 11, 1726–1732 (1972). For instance, Abu, Ala, Gly, Cys, Lys, Asn, Asp, Phe, Trp, L-Trp, D-Trp, DL-Trp, D-5-Br-Trp, L-5-F-Trp, Thr and Ser represent the "residues" of α-aminobutyric acid, L-alanine, glycine, L-cysteine, L-lysine, L-asparagine, L-aspartic acid, L-phenylalanine, L-tryptophan, L-tryptophan, D-tryptophan, DL-tryptophan, D-5-bromotryptophan, L-5-fluorotryptophan, L-threonine and L-serine, respectively. The term "residue" refers to a radical derived from the corresponding alpha-amino acid by eliminating the hydroxyl of the carboxyl group and one hydrogen of the alpha-amino group.

SUMMARY OF THE INVENTION

The present invention relates to analogs of the tetradecapeptide somatostatin. More particularly, this invention relates to octapeptide somatostatin analogs of the formula I:



(I)

wherein

A is an L, D or DL amino acid selected from the group consisting of Alanine (Ala), valine (Val), phenylalanine (Phe), para-chloro-phenylalanine (p.Cl Phe), tryptophan (Trp), proline (Pro), serine (Ser), Threonine (Thr), tyrosine (Tyr), glutamic acid (Glu), beta alanine (Beta Ala), α-aminobutyric acid (Abu), N-methylalanine (N-Me Ala), 5-fluorotryptophan (5-F Trp), 5-bromotryptophan (5-Br Trp), 5-chlorotryptophan (5-Cl Trp), their acetylated derviatives or a pharmaceutically acceptable acid addition salt thereof;

B is an L, D or DL amino acid selected from the group consisting of threonine amide (Thr NH_2), valine amide (Val NH_2), proline amide (Pro NH2), hydroxyproline amide (HO Pro NH_2), serine amide (Ser NH2), tyrosine amide (Tyr NH_2), tryptophan amide (Trp NH2), 5-fluorotryptophan amide (5-F Trp NH_2), formyl tryptophan amide (For Trp NH), alanine amide (Ala NH_2), glycine amide (Gly NH2) and methylalanine amide (Me Ala NH_2);

X is L-phenylalanine (L-Phe) or L-tyrosine (L-Tyr);

Y is L-threonine (L-Thr) or L-valine (L-Val);

Z is L, D or DL 5-halo-tryptophan, in which the halogen (Halo-) is fluorine, chlorine, bromine or iodine, or D-Tryptophan (D-Trp); and

C'' and C' are L or D-cysteine (Cys), α-aminobutyric acid (Abu), aspartic acid (Asp) or lysine (Lys); and the pharmaceutically acceptable acid addition salts thereof. Certain derivatives are within the scope of this invention. Thus Cys alone means D-cysteine in the sulfhydryl form, whereas a bridge between two cys groups should be read as a disulfide bridge unless there is any supplemental indication. MBz is p-methoxy benzyl, 2-ClZ is 2-chlorocarbobenzyloxy, similarly 2-BrZ is the corresponding 2-bromocarbobenzyloxy group. Where such groups appear next to an amino acid, either prior or subsequent, but between hyphens, such a group is a substituent on the aminoacid to which it is adjacent.

Further, the present invention encompasses the novel intermediates which are the reduced forms of the compounds of formula I and methods and compositions utilizing these novel octapeptides for the treatment of various mammalian disorders.

These octapeptides inhibit the release of such hormones as growth hormone, prolactin, insulin, glucagon, gastrin, secretin and cholecystokinin, as well as diminish gastrin-stimulated secretion of gastric acid. These effects may be independent of the administration of other physiologically active compounds or as an effect of the combination of the subject composition with other physiologically active compounds.

The octapeptides of the present invention can thus be used for the treatment of such disease states as diabetic retinopathy, diabetes, ulcers, acute pancreatitis and acromegaly.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The octapeptides of the present invention are encompassed by the above formula I. These octapeptides exist in a cyclic form due to a bridge between the C'' and C' substituents. This bridge may be a disulfide bridge (—S—S—), a carbon/sulfur bridge (—C—S—), a carbon/carbon bridge (—C—C—) or an amido bridge (—CO—NH—) depending on the method of formation. The reduced forms of the octapeptides of formula I are encompassed by formula I' below. These reduced forms are intermediates in the process for preparing the compounds of formula I.

Of the compounds of formula I, certain combinations of substituents are preferred. For instance, compounds wherein C'' is Cys, X is Phe, Z is D-Trp, Y is Thr, and C' is Cys; compounds wherein C'' is Cys, X is Tyr, Z is D-Trp, Y is Val and C' is Cys; X is Phe, Y is Thr, and C' is Cys; and compounds wherein C'' is Cys, X is Tyr, Y is Val, and Cys are preferred compounds.

The octapeptides of this invention are obtainable in the form of the free base or in the form of a pharmaceutically or therapeutically acceptable acid addition salt. The octapeptides in the form of the free bases are readily obtainable from the corresponding acid addition salt by conventional methods, for example, a solution of the acid addition salt is passed through an anionic exchange resin (OH form) to obtain the free base. The free base can also be obtained from the acetic addition salt by repeated lyophilization of the latter salt from aqueous solution. The acetic acid addition salt is readily obtainable from another acid addition salt by treatment with the apropriate ion exchange resin, for example, Sephadex G-15 using 50% acetic acid in the manner

described by Coy, et al., *Biochem. Biophys. Res. Commun.*, 1267–1273 (1973).

The octapeptides of this invention can be obtained in the form of a pharmaceutically or therapeutically acceptable acid addition salt either directly from the process of this invention or by reacting the peptide with one or more equivalents of the appropriate acid. Examples of preferred non-toxic salts are those with pharmaceutically or therapeutically acceptable organic acids, e.g., acetic, lactic, succinic, benzoic, salicyclic, methanesulfonic, toluenesulfonic or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose and salts with inorganic acids such as the hydrohalic acids, e.g., hydrochloric acid or sulfuric acid or phosphoric acid.

The octapeptides of this invention can be prepared by solid phase synthesis. The synthesis begins at the C-terminal end of the peptide. The first protected amino acid is linked to the benzhydrylamine resin by reaction of the carboxyl group protected amino acid in the presence of N,N'-dicyclohexylcarbodiimide or N,N'-diisopropylcarbodiimide and 1-Hydroxybenzotriazole (HOBT). The sequential building of the peptide involves the stepwise addition of each amino acid in the N-terminal portion of the peptide chain.

The cleavage of the N-terminal protecting group is accomplished by using trifluoroacetic acid. The other protecting groups present on the peptide chain are stable under the conditions of the cleavage of the N-terminal protecting group. Once the N-terminal deprotection has been effected, the product which results normally will be in the form of the addition salt of trifluoroacetic acid. The free terminal amino compound is formed by treating with a mild base, typically a tertiary amine such as triethylamine or diisopropylethylamine. The peptide resin is then ready for the coupling with the next amino acid which has a free carboxyl but which is protected at the alpha-amino group.

Once the desired amino acid sequence is prepared, the resulting peptide is removed from the resin support. This is accomplished by the treatment of the peptide resin with hydrogen fluoride. The hydrogen fluoride cleaves the peptide from the resin and, in addition, it cleaves all remaining protecting groups except formyl group of Trp. This treatment of hydrogen fluoride is carried out in the presence of m-cresol and anisole which are found to inhibit the potential alkylation of certain amino acids present in the peptide chain.

When the cleavage reaction is accomplished, the product is obtained in the reduced form, i.e., of the formula I':

A-C''-X-Z-Lys-Y-C'-B (I')

wherein A, C'', X, Z, Y or C' and B are as hereinbefore defined. Oxidation will generate a disulfide bridge and allow the products to be obtained in the cyclized form of formula I. Use of well known coupling techniques will yield, in place of a disulfide bridge, an amido bridge between C'' and C'.

Although the selection of the particular protecting groups to be employed in preparing the compounds of this invention remains a matter well within one ordinarily skilled in the art, it should be recognized that the proper selection of the protecting groups is dependent upon the particular succeeding reactions which must be carried out. Thus, the protecting group of choice must be one which is stable both to the reagents and under the conditions employed in the succeeding steps of the reaction sequence. For example, as already discussed hereinabove, the particular protecting group employed must be one which remains intact under the conditions which are employed for cleaving the alpha-amino protecting group of the terminal amino acid residue of the peptide fragment in preparation for the coupling of the next succeeding amino acid fragment to the peptide chain. It is also important to select as protecting group, one which will remain intact during the building of the peptide chain and which will be readily removable upon completion of the synthesis of the desired octapeptide product. All of these matters are well within the knowledge and understanding of one ordinarily skilled in the art.

The octapeptides produced by the process of this invention, as well as their corresponding pharmaceutically or therapeutically acceptable acid addition salts, are useful due to their possession of the pharmacological activity of the natural tetradecapeptide somatostatin. Such activity is demonstrated readily in pharmacological tests such as a modification of the in vitro method of Saffran and Schally, *Can. J. Biochem. Physiol.*, 33, 405 (1955) as given in Schally, et al., *Biochem. Biophys. Res. Commun.*, 52, 1314 (1973) and River, et al., *C.R. Acad. Sci. Paris*, Ser. D., 276, 2337 (1973).

The ability of the octapeptides of this invention to inhibit hormone release in vitro is demonstrated by the method described by Meyers, et al., *Biochem. Biophys. Res. Commun.*, 74, 630 (1977). In this method, the octapeptides of this invention are shown to inhibit the release of radioimmunoassayable growth hormone and prolactin in vitro from enzymatically dispersed rat anterior pituitary cells prepared as described by Labrie, et al., *Sixth Karolinska Symp. on Res. Meth. in Reprod. Endocrinol.*, (E. Diczfalusy, Ed.). pp 301–328 (1973). Following four days in culture, the cells are washed and incubated for five hours at 37° C. in Dulbecco-modified Eagle's medium in the presence or absence of increasing concentrations of each octapeptide analog. Growth hormone and prolactin levels are determined by radioimmunoassay, using methods described by Birge, et al., *Endocrinol.*, 81, 195–204 (1967) for rat growth hormone and Niswender, et al., *Proc. Soc. Exp. Biol. Med.*, V 130, 793 (1969) for prolactin. NIAMDD Rat GH and prolactins RIA kits here used as standards. The dose required for a 50% inhibition of growth hormone release (ED 50) is calculated for each analog by the method of Rodbard, *Endocrinol.*, 94, 1427–1437 (1974).

In vitro inhibition of growth hormone and prolactin is also measured in a pituitary cell superfusion system as described by Vigh and Schally (Peptides, Vol. 5, Suppl. 1., p. 241–247). Adult male or female Sprague-Dawley strain rats are decapitated for each experiment. The anterior pituitaries are cut into small pieces and incubated in a Dubnoff incubator for 45 minutes at 37° C. in 10 ml. of oxygenated Medium 199 (GIBCO) containing 0.5% collagenase, 0.25% bovine serum albumin (BSA), and 50 ul/ml Gentamicin Sulphate. After this incubation, the fragments can be easily dispersed into single cells by repeated suction and expulsion from a Gilson Pipetman. After 30 to 60 Pipetman operations, the tissue falls apart. The cell suspension is centrifuged at room temperature for 10 minutes at 100 g. The cell pellet is then resuspended in 1.0 ml. of medium. A small aliquot is diluted for counting the cells and the rest of the suspension is divided into 4 equal volumes. Each volume

(containing about 5×10^6 cells) is mixed with 0.5 ml. Sephadex G-15 which has been equilibrated with previously oxygenated medium. The mixture of pituitary cells and Sephadex is transferred into four chambers of the superfusion apparatus consisting of a number of 1 ml. plastic syringe barrels (modified by cutting off their distal end) and mounted vertically in a plexiglas holder which was kept at 37° C. by circulating water. The flow through the system (0.5 ml. min) is controlled with a multichannel peristaltic pump (Vigh and Schally, Peptides, Vol. 5, Suppl. 1, p. 214–247. Rat prolactin and rat GH were measured in aliquots of superfusates by RIA to determine either basal or inhibited secretion.

The in vivo growth hormone bioassay utilized is as follows:

Male Charles River CD rats (200–300 g.) with free access to food and water were anesthetized with sodium pentobarbital (60 mg.kg intraperitoneally). Thirty minutes later saline or octapeptide is injected subcutaneously and blood samples are drawn from the jugular vein 15 minutes after subcutaneous injection. The plasma is separated and assayed for growth hormone by RIA as described above. This in vivo bioassay is a modification of the methods used by Schally, et al., "Hypothalamic Peptide Hormones: Basic and Clinical Studies", "*Hormonal Proteins and Peptides*", C. H. Li, ed. Academic Press, N.Y. 7:1–54, 1978; Meyers, C. A., Murphy, W. A., Redding T. W., Coy, D. H. and Schally, A. V., *Proc. Natl. Acad. Sci., USA* 77:6171, 1980; Murphy, W. A., Meyers, C. A. and Coy, D. H., *Endoc.* 109: 491, (1981) as well as Veber et al., *Pro. Natl. Acad. Sci.*, USA 75:2636–2640 (1978). For some analogs, doses as low as 0.02 ug/100 g. or even 0.005 ug/100 g. are active. The potency of the octapeptides of this invention relative to somatostatin is illustrated in Tables A, B and C. Single dose assays are shown in Table A and 4 point assays at 2 dose levels in Tables B and C.

TABLE A

Single Dose Assays of Somatostatin Octapeptide Analogs vs. Somatostatin 14. (SS-14)Inhibition of GH Release.

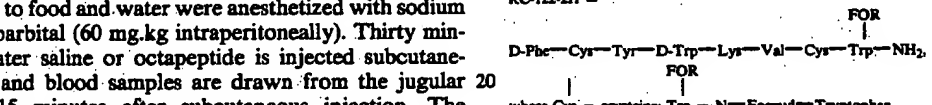
SUBSTANCE TESTED	AMOUNT INJECTED/ 100 g.B.W. (μg)	GH-LEVEL (ng/ml) mean ± SEM
RC-121-2H	0.05 μg	61 ± 17*
RC-114-2H	0.05 μg	55 ± 10*
SS-14	2.00 μg	95 ± 31*
CONTROL	SALINE	754 ± 155
RC-122-2H	0.20 μg	23 ± 3*
SS-14	2.00 μg	50 ± 12*
CONTROL	SALINE	374 ± 69

*p (0.01 vs Control (Duncan's test).

RC-121-2H =
D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Thr—NH_2

RC-114-2H =
D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Ser—NH_2

RC-122-2H =



where Cys = cysteine; Trp = N—Formyl—Tryptophan

TABLE B

Four-point Assay of GH-Release Inhibition Activity of Somatostatin Octapeptides Analogs vs. Somatostatin-14 in rats.

SUBSTANCE TESTED	AMOUNT INJECTED/ 100 g.b.w. (μg)	GH-LEVEL (ng/ml) mean ± SEM
CONTROL	SALINE	277 ± 166
SS-14	0.4 μg	86 ± 16
SS-14	1.6 μg	47 ± 17
RC-121-2H	0.02 μg	39 ± 10
RC-121-2H	0.08 μg	21 ± 4
RC-114-2H	0.02 μg	44 ± 11
RC-114-2H	0.08 μg	34 ± 9

RC-121-2H vs SS-14: M = 0.7709, Antilog M = 5.9, Potency = 118-times vs SS-14
Rc-114-2H vs SS-14: M = 0.6757, Antilog M = 4.74, Potency = 95.8-times vs SS-14
RC-121-2H = D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Thr—NH_2
RC-114-2H = D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Ser—NH_2
Factorial statistical analyses and calculation of potencies in 4 point design assays were carried out by the method of Bliss and Marks (Bliss, C.I. and Marks, H.P. : Quart.J.Pharm. and Pharmacol. 12,82 and 182, 1946), and Pugsley, (L.I. : Endocr., 39, 161–176, 1946).

TABLE C

Relative Potencies of Somatostatin and Somatostatin Analogs on Inhibition of GH and Insulin Release in vivo based on 4-point Assays.

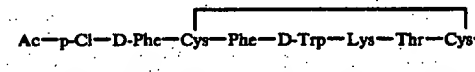
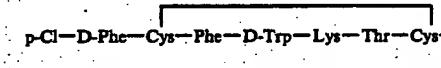
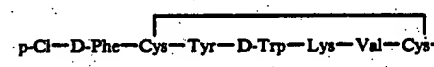
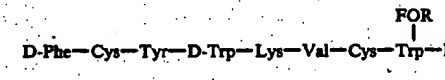
CODE	PEPTIDE SS-14 (Somatostatin)	INHIBITION POTENCY GH in vivo 100	INSULIN in vivo 100
RC-15		860	
	Ac—p-Cl—D-Phe—Cys—Phe—D-Trp—Lys—Thr—Cys—Thr—NH_2		
RC-76-2H	Ac—D-Phe—Cys—Phe—D-Trp—Lys—Thr—Cys—Pro—NH_2	1070	
RC-127-2H	D-Val—Cys—Phe—D-Trp—Lys—Thr—Cys—Thr—NH_2	790	
RC-138-2H	D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Ala—NH_2	1570	
RC-88		2130	
	p-Cl—D-Phe—Cys—Phe—D-Trp—Lys—Thr—Cys—Thr—NH_2		
RC-88-II-2H	p-Cl—D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Thr—NH_2	3020	1860
RC-88-II		1740	
	p-Cl—D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Thr—NH_2		
RC-122-2H		5540	
	D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Trp—NH_2		
RC-114-2H	D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Ser—NH_2	9580	400
RC-121-2H	D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Thr—NH_2	11800	

TABLE C-continued

Relative Potencies of Somatostatin and Somatostatin Analogs on Inhibition of GH and Insulin Release in vivo based on 4-point Assays.

CODE	PEPTIDE SS-14 (Somatostatin)	INHIBITION POTENCY GH in vivo 100	INSULIN in vivo 100
RC-121	D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Thr—NH_2	19900	910
RC-101-II-2H	Ac—D-Phe—Cys—Phe—D-Trp—Lys—Thr—Cys—Thr—NH_2	12900	
RC-101-I-2H	Ac—Phe—Cys—Phe—D-Trp—Lys—Thr—Cys—Thr—NH_2	11320	
RC-159-II	D-Phe—Cys—Tyr—Trp—Lys—Val—Cys—Thr—NH_2	2380	
RC-160	D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Trp—NH_2	13400	
RC-122	D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Trp—FOR	4980	
RC-113-2H	D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Tyr—NH_2	3020	
RC-161	Ac—D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Thr—NH_2	5520	
RC-101-I	Ac—Phe—Cys—Phe—D-Trp—Lys—Thr—Cys—Thr—NH_2	13560	
RC-95-I	D-Phe—Cys—Phe—D-Trp—Lys—Thr—Cys—Thr—NH_2	5280	

Factorial statistical analyses and calculation of potencies in 4 point design assays were carried out by the method of Bliss and Marks (Bliss, C. I. and Marks, H. P.: Quart J. Pharm. and Pharmacol. 12, 82 and 182, 1939) and Pugsley (Pugsley, L. I., Endocr., 39, 161–176, 1946).

In order to determine the time course of the compounds of this invention, male rats are anesthetized with Numbutal and injected with saline or the test octapeptide as in the growth hormone potency assay above. Blood is collected from the jugular vein 15, 30, 45, 60, 90, 120 and 180 minutes post subcutaneous above. This time-course experiment for octapeptides on growth hormone release in vivo a significant suppression of plasma growth hormone. At a dose of 0.1 ug/100 g. administrated subcutaneously, the octapeptide

D—Phe—Cys—Tyr—D—Trp—Lys—Val—Cys—ThrNH$_2$

significantly inhibits growth hormone release for at least 3 hours. At the dose of 2 ug/100 g., the inhibitory effect of somatostatin on growth hormone release is no longer observed after 30 minutes (FIG. 1). The synthetic octapeptides also suppressed growth hormone and prolactin levels in vitro.

The octapeptides of the present invention are also compared to synthetic somatostatin for their ability to inhibit the release of insulin and glucagon in vivo in male rats (CD strain, Charles River) weighing 250–300 g. The rats are kept in controlled temperature (24° C.) and light (0500–1900h) conditions for 1 week before the assay. Rats are fasted 27–30 hours with free access to water then anesthetized with Nembutal (6 mg/100 g. intraperitoneally). After 30 minutes, saline or test peptide is injected into the jugular vein and blood is collected 5 minutes later from the hepatic portal vein then transferred into chilled tubes containing EDTA (2.5 mg/ml.) and Trasylol (500 K.I.U./ml.). Plasma is separated and stored at 31 20° C. until assayed for insulin and glucagon. Plasma insulin is determined by double antibody radioimmunoassay using a kit from Cambridge

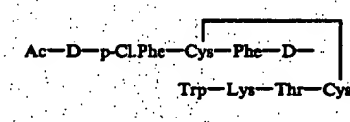
Plasma glucagon is determined by the method of Faloona, et al., (1974) In: Methods of Hormone Radioimmunoassay, Eds. Jaffe, B. and Behrman, H. R., Academic Press, New York, pp. 317–330, using crystalline glucagon (Eli Lilly) and rabbit antiserum 30K against glucagon (Unger pool 4, lot 8). Porcine ^{125}-glucagon was also purchased from Cambridge Nuclear Radiopharmaceutical Corp., Mass.

In the test systems described above, the octapeptides of the present invention were found to inhibit insulin release in vivo more powerfully than somatostatin on a weight basis (see Table C). Because of the high potency of the octapeptides of the present invention, the absence of toxicity and the long duration of their activity, these octapeptides are useful for application in the treatment of a number of diseases and conditions. For example, the octapeptides of the present invention can be used to assess insulin resistance in obese patients. The use of this compound prevents endogenous insulin secretion. Previously, somatostatin or propranolol were used for this purpose. (Shen, et al., *J. Clin. Invv.*, 49, 2151 (1970)). In this method, to demonstrate total body resistance to exogenous insulin in obese subjects, standard quantities of glucose, insulin and octapeptides are infused for 150 minutes. A steady state level of plasma insulin and glycose should be attained after 90 minutes. Endogenous

insulin secretion determined by C-peptide measurement and glucagon secretion remains suppressed throughout the period. With steady state levels of plasma insulin maintained in the subjects, the height of the steady state plasma glucose concentration can be considered an index of total body sensitivity to insulin mediated glucose uptake. A positive correlation between steady state plasma glucose concentration and the degree of obesity can be demonstrated. This is supported by similar studies with somatostatin of Magulesparan et al., *Diabetes*, 28, 980 (1979), but the octapeptides are superior due to their prolonged suppression of insulin.

In diabetic patients who do not produce insulin, by inhibiting growth hormone and glucagon secretion by employing the octapeptides of the present invention, the dose of insulin should be reductible to ¼–⅛ of that required by the patient in the absence of octapeptides. This is supported by the work of Besser, et al., *Brit. Med. Journal* 4, 622–627 (1974) and Gerich, et al., *Diabetologia*, 13, 537 (1977). These studies utilized somatostatin as an adjunct to insulin. Similarly, the octapeptides of the present invention can advantageously be used in place of somatostatin. In addition, the octapeptides are also useful for treatment of diabetic retinopathy by inhibiting growth hormone secretion which causes vascular damage.

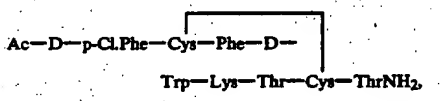
In some patients, after removal of the hypophysis and after bromocryptine treatment, an elevated growth hormone level is still observed. Utilizing the octapeptides of the present invention, in combination with bromocryptine or another similarly effective drug, the growth hormone level is reduced. In addition, LHRH agonists, which given chronically lead to paradoxical inhibitory effects, can also be used. A combined hormonal treatment consisting of octapeptides, LH-RH analogs and bromocryptine can be used for the treatment of acromegaly, while similar combination treatments are useful for other neoplasmas. When the tumors are hormone dependent and these hormones are inhibited by octapeptides, these tumors can be treated with octapeptides. For example, murine and other mammalian chondrosarcomas are growth hormone dependent. Salomon, et al., *Cancer Res.*, 39, 4387 (1979); and McCumbee, *Fed. Proc.*, 30, 428 (1979) reported that rat (Swarm) chondrosarcomas are growth hormone dependent. Human chondrosarcomas are similar to Swarm chondrosarcomas. Somatostatin analogs inhibit the growth of swarm chondrosarcomas (Redding and Schally, A.V. Proc. Nat. Acad. Sciences, 80, 1078–1082 (1983)) and the octapeptides of the present invention are likewise useful for inducing the regression of human chondrosarcoma tumors which cannot be controlled by chemotherapy. Similarly, Dunn-Osteosarcomas C3H/HeJ in mice are similar to human tumors and appear to be hormonally (GH)-dependent (Ghanta et al., *Natl. Cancer Inst.* 57, 837–839 (1976)). Various analogs of somatostatin inhibit the growth of Dunn osteosarcomas (Schally, et al., *Cancer Treatment Reports*, 68, 281, (1984)), Schally, et al., *Proc. Soc. Exp. Med.*, 175, 259 (1984). Octapeptide RC-15,



at doses of 5 ug/day was found to significantly prolong the survival of mice with Dunn osteosarcomas. Octapeptide RC-121-2H, D-Phe-Cys(SH)-Tyr-D-Trp-Lys-Val-Cys(SH)-ThrNH$_2$, in doses of 2.5 ug/ b.i.d. also significantly inhibited growth of Dunn's osteosarcoma tumors in C3H female mice. The % survival was 100%±0.0% as compared to 75%+22% for controls by day 16.

The measurement of antitumor activity of the octapeptides of this invention in an animal mammary cancer model is as follows:

Several hormone-dependent mammary tumors in rats and mice have been shown to be estrogen dependent and/or prolactin dependent (Arafah et al., *Endocrinology*, 107, 1364 (1980)); Schally, et al., *Proc. Soc. Exp. Biol. Med.*, 175, 259 (1984). It is known that about ⅓ of human breast cancers are estrogen dependent. Recent evidence indicates that a significant proportion (30–40%) of human breast cancers may also prolactin dependent (Malarkey, et al., *J. Clin. Endoc. Metab.*, 56, 673–677 (1983)); Ben, et al., *Israeli J. Med. Sci.*, 17,965 (1981). In addition, growth hormone is also involved in the growth of breast cancers. The octapeptides of the present invention, by inhibiting prolactin and growth hormone secretion, may be useful for inducing regression of human breast carcinomas. The antitumor activity of the octapeptides of the present invention are measured in the MT/W9A rat mammary adenocarcinoma model. The estrogen-dependent MT/W9A rat mammary adenocarcinoma is obtained from Roswell Park Memorial Institute, Buffalo, N.Y. The MT/W9A mammary tumor has been characterized as estrogen-dependent and it needs physiological levels of both prolactin and estrogen for growth (Kim and Depowski, *Cancer Research*, 35, 2068–2077 (1975)). Tumor tissue is minced into approximately 1 mm^3 pieces, or until a fine slurry has been made, and injected through an 18 G needle into the inguinal mammary fat pad of female Wistar-Furth rats (90 gms. body weight). After about 3½ months the tumors were palpable and the experiment is initiated. Octapeptide RC-15,



in doses of 3 ug/b.i.d. (twice a day) powerfully inhibited the growth of MT/W9A rat mammary tumor and reduced the percentage change in tumor volume to 1106±447% as compared to 2808±812% for controls, (FIG. 2) after 16 days of treatment. Thus, the octapeptides of the present invention may be utilizable in the treatment of human breast carcinoma. The therapeutic action of octapeptides is due to the inhibition of the growth hormone and prolactin secretion. The octapeptide may be administered alone or in combination with LH-RH analogs such as D-Trp-6-LH-RH, which inhibits the growth of estrogen dependent mammary tumor (Redding and Schally, *Proc. Natl. Acad. Sci.*, USA, 80, 1459–1462 (1983)).

In order to determine activity on pancreatic carcinoma, animal models of pancreatic cancer with acinar and ductal phenotypic characteristics are used. (Redding and Schally, *A.V., Proc. Nat. Acad. Sci.*, 81, 248–252 (1984)). The transplantable well differentiated acinar pancreatic tumors DNCP-322 (CA-20948) in Wistar/Levis rats (Longnecker, et al., *Cancer Research*, 42, 19–24, (1982)); and Longnecker, et al., *Cancer Let-*

ters, 7, 197–202 (1979) are obtained from the Department of Pathology, Dartmouth Medical School, Hanover, N.H. The golden Syrian hamsters bearing the well differentiated (WD), chemically induced ductal adenocarcinoma (Scarpelli, et al., *Cancer Res.*, 39, 452–458 (1979) are used. Donor tumor tissue from either species is quickly dissected and washed in ice cold Hanks buffered saline, pH 7.4 and the capsular material carefully removed. Tumor tissue is then sliced into small pieces and passed through a No. 30 stainless steel screen into a beaker of ice cold buffer. The pellet is resuspended in buffer and 1 to 2 mg. aliquots of tumor tissue injected subcutaneously into the middle back region of weanling male animals of the respective model, i.e., Wistar/Levis rats of LAS Syrian hamsters. Subcutaneously transplanted Longnecker DNCP-322 tumors achieve a diameter of approximately 5 cm in about 4 weeks. WD tumor grows slowly in golden hamsters, tripling its size in about 45 days. In rats bearing the acinar pancreatic tumors, chronic administration of the octapeptides of this invention significantly decreased tumor weights and volume. In Syrian hamsters bearing ductal form of pancreatic cancer, chronic administration of the octapeptides diminished tumor weights and volume (Redding and Schally, *Proc. Nat. Acad. Sci.*, 81, 248–252 (1984)). The percentage change in tumor volume volume was significantly decreased when compared to control animals. The LHRH agonist D-Trp-6LH-RH, given twice daily or injected in the form of controlled-release microcapsules significantly decreased tumor weight and volume. The octapeptides reduce the growth of pancreatic ductal and acinar cancers, probably by inhibiting the relase and/or stimulatory action of gastrointestinal hormones, gastrin, secretin and cholecystokinin on tumor cells (Schally, et al., *Proc. Soc. Exp. Biol. Med.*, 175, 259 (1984)). A combined administration of an octapeptide of this invention with an LH-RH agonist leads to a greater inhibition of cancers of the pancreas than that which can be obtained with somatostatin analogs alone.

In order to compare some of the biological actions of the octapeptides of the present invention and somatostatin on gastrointestinal secretions and on the release of some gastrointestinal homones in dogs, the following procedures are used:

Mongrel dogs weighing 15–20 kg. are prepared surgically with gastric (GF) and pancreatic fistulae (PF) as described by Konturek, et al., (1976) 225, 497 and Konturek, et al., *Gastroenterology*, (1976) 58, 1–6. Secretions from the GF and PF are collected continuously at 15 minute intervals. Hydrogen ion and pepsin concentrations in the pancreatic juice are also measured in 15- or 30-minute outputs. (Konturek, et al., *J. Physiol. Lond.*, 255, 497 (1976) and Konturek, et al., (1976) supra.). Basal secretion is first collected for two 15 minute periods, and then the secretory stimulant for gastric and/or pancreatic secretion is administered for 3½ hours. When the secretory rate reaches a subtained plateau, the octapeptides or somatostatin are given in standard doses (2–4 ug/Kg/hr.). i.v. for a one hour period. In control experiments, the animals received secretory stimulants along for the duration of the tests.

In tests on gastric stimulation, pentagastrin or desglugastrin (glutaroyl-Ala-Tyr-Gly-Trp-Leu-Asp-Phe-NH_2) is infused i.v. in a constant does (3 ug/kg/hr.), shown previously to elicit near maximal gastric acid secretion. Gastic secretion is measured and acid content determined by titration with 0.1N NaOH. In tests on pancreatic secretion, synthetic secretion (Squibb and Sons, Inc., New York, N.Y.) and caerulein (Farmitalia, Italy) are used in constant doses shown previously to evoke near maximal stimulation of bicarbonate of pancreatic enzyme secretion (Konturek, et al., (1976) supra.

Experiments on pancreatic stimulation by endogenous stimulants are performed using duodenal instillation of 0.1N HCl or a meat meal (500 g.), (Konturek, et al., (1976) supra). Blood samples are withdrawn before and every 15–20 minutes for serum gastrin determination (Yalow, et al., 58, 1–14 (1970).

The results observed for the octapeptides of the present invention and somatostatin on gastric acid response to pentagastrin are as follows:

Both the octapeptides and somatostatin administered are found to strongly inhibit pentagastrin or desglugastrin-induced gastric acid output fom the GF. The inhibition of acid secretion is more pronounced with the octapeptides. D-p-Cl.Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-ThrNH_2 was found to be approximately twice as potent as somatostatin. Moreover, its action was protracted since the inhibition of gastric acid continues after infusion was stopped, in contrast to somatostatin whose action was short-lived.

G.I. tests indicate that the octapeptides are useful for treatment of duodenal ulcers and acute pancreatitis. The octapeptides of this invention or the acid addition thereof are also useful for the treatment of acromegaly and related hypersecretory endocrine states and in the management of diabetes and for the therapy of certain cancers. When the octapeptides or salts thereof are employed for such treatment or management, they are administered systemically, preferably parenterally, or in combination with a pharmaceutically acceptable liquid carrier. The octapeptides of this invention have a low order of toxicity. The proportion of the octapeptide or salt thereof is determined by its solubility in the given carrier, by the given carrier, or by the chosen route of administration. When the octapeptide or a salt thereof is used in a sterile solution, such solution may also contain other solutes such as buffers or preservatives, as well as sufficient amounts of pharmaceutically acceptable salts or glucose to make the solution isotonic. The dosage will vary with the form of administration and with the particular species to be treated. Preferably, the dose range for sublingual or oral administration is about 1 mg. to about 100 mg/kg. of body weight per day. Generally, the dose range for intravenous, subcutaneous or intramuscular administration is from about 0.1 mcg. to about 1 mg/kg. of body weight per day, and, preferably, is from about 0.5 mcg. to about 100 mcg./kg. of body weight per day. It is evident that the dose range will vary widely dependent upon the particular condition which is being treated as well as the severity of the condition.

The octapeptides or salts thereof can be also be administered in one of the long-acting, slow-releasing or depot dosage forms described below, preferably by intramuscular injection or by implantation. Such dosage forms are designed to release from about 0.1 mcg. to about 50 mcg./kg. body weight per day.

It is often desirable to administer the octapeptide continuously over prolonged periods of time in long-acting, slow-release or depot dosage forms. Such dosage forms may either contain a pharmaceutically acceptable salt of the peptide having a low degree of solubility in body fluids, for example, one of those salts described below or they may contain the peptide in the

form of a water-soluble salt with a protective carrier which prevents rapid release. In the latter case, for example, the peptide may be formulated with a non-antigenic partially hydrolyzed gelatin in the form of a viscous liquid; or the peptide may be absorbed on a pharmaceutically acceptable solid carrier, for example, zinc hydroxide, and may be administered in suspension in a pharmaceutically acceptable liquid vehicle; or the peptide may be formulated in gels or suspensions with a protective non-antigenic hydrocolloid, for example, sodium carboxymethylcellulose, polyvinylpyrrolidone, sodium alginate, gelatin, polygalacturonic acids, for example, pectin, or certain mucopolysaccharides, together with aqueous or non-aqueous pharmaceutically acceptable liquid vehicles, preservatives, or surfactants. Examples of such formulations are found in standard pharmaceutical tests, e.g., in Remington's Pharmaceutical Sciences, 14th. Ed., Mack Publishing Co., Easton, Pa. (1970). Long-acting, slow-release preparations of the octapeptides of this invention may also be obtained by microencapsulation in a pharmaceutically acceptable containing, for example, gelatin, polyvinyl alcohol or ethyl cellulose, or co-polymers of lactic and glycolic acids, poly(d,l-lactide-co-glycolide) microcapsules, cf. T. W. Redding, et. al., *Proc. Natl. Acad. Sci.*, USA, 81, 5845–5848 (1984)) and the like.

Further examples of coating materials and of the processes used for microencapsulation are described by J. A. Herbig in "Encyclopedia of Chemical Technology", Vol. 13, 2nd Ed., pp. 436–456 (1967) Wiley, New York. Such formulations, as well as suspensions of salts of the peptide which are only sparingly soluble in body fluids, for example, salts with pamoic acid or tannic acid, are designed to release from about 0.1 mcg. to about 100 mcg. of the active compound/kg. body weight per day, and are preferably administered by intramuscular injection. Alternatively, some of the solid dosage forms listed above, for example, certain sparingly water-soluble salts or dispersions in or absorbates on solid carriers of salts of the peptides, for example, dispersions in a neutral hydrogel of a polymer of ethylene glycol methacrylate or similar monomers cross-linked as described in U.S. Pat. No. 3,551,556, may also be formulated in the form of pellets releasing about the same amounts as shown above and may be implanted subcutaneously or intramuscularly.

The invention will appear more fully from the examples which follow. These examples are set forth by way of illustration only and it will be understood that the invention is not to be construed as limited either in spirit or in scope by the details contained therein as many modifications, both in materials and methods will be apparent to those skilled in the art. Throughout these examples the following purification techniques are utilized:

(Abbreviations:
HFBA=heptafluorobutyric acid
HPLC=high-performance liquid chromatography
TFA=tricluoroacetic acid
UV=ultraviolet)

For preparing the HPLC elements, UV grade acetonitrile is purchased from Burdick & Jackson, TFA and HFBA are of Sequanal grade from Pierce and water is double-distilled in glass and passed through a Milli-Q system (Millipore).

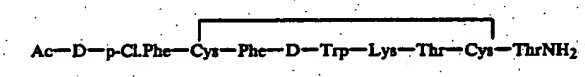
High-performance liquid chromatography is carried out on a Waters HPLC system consisting of an M 680 Automated Gradient controller, two M6000A pumps, a U6K injector and a Schoeffell SF 770 variable wavelength UV detector.

Reversed phase HPLC on C18 columns is used both for analyzing and purifying the compounds. The quality and the elution characteristics of the crude peptides are established by analytical HPLC on a Vydac 218TP5 column (4.6 mm×25 cm) using binary gradients of solvent A: 0.1% TFA in water, and solvent B: 0.1% TFA in CH_3CN/water 70:30. The good quality of the crude synthetic products combined with optimized separation conditions allows a rapid, one-step purification scheme. Resolution comparable to that of analytical separations was achieved by using a 5 um particle size packing material and a sample load below the capacity of the column. Six to twenty-three mg. of octapeptide is injected in 2–5 mg. portions onto a semi-preparative Vydac 218TP5 column (10 mm×25 cm) and eluted isocratically or by a flat gradient (0.1–0.2% B/min) using the solvent system containing 0.1% TFA, as described below.

For optimal peak "shaving" the main components are collected manually. The volatile eluent is removed by freeze drying and then the products are re-lyophilized from 1M AcOH yielding 0.7 mh (10–45%) of the purified octapeptide. The homogeneity of the octapeptides is checked by analytical HPCL in two different solvent systems (I: 0.1% TFA/CH_3CN/water, II: 0.13% HBA/CH_3CN/water). Purity using these techniques is better than 90% based on UV absorbances monitored at 210 nm.

EXAMPLE 1

Ac—D—p-Cl.Phe—Cys—Phe—D—Trp—Lys—Thr—Cys—ThrNH$_2$ and



0.50 g. benzhydrylamine (BHA) resin (ca. 0.5 NH_2/g. resin) is added to a 10 ml. reaction vessel with a special fritter filter of medium porosity, treated with 10% triethylamine in CH_2Cl_2 two times each for three minutes and washed with CH_2Cl_2 six times.

The resin is mixed with Boc-Thr(Bz) (0.75 m. moles) and HOBt (0.82 m. moles) in DMF for three minutes. 5% diisopropylcarbodiimide (0.82 m. moles) CH_2Cl_2 is added. The mixture is shaken at room temperature for 90 minutes. The resulting resin is washed with CH_2Cl_2 six times and is subjected to a ninhydrin test (Kaiser, et al., *Analytical Biochemistry*, 34, 595 (1970)). It should be negative at this stage.

The deprotection of the Boc- group from Boc-Thr(Bz)-BHA resin is carried out as follows: The resin is treated with a solution of trifluoroacetic acid and methylene chloride (1:1) for 5 minutes, filtered and treated again for 25 minutes, filtered and then washed with CH_2Cl_2 six times.

Treatment with 10% triethylamine is performed as described for the benzhydrylamine resin.

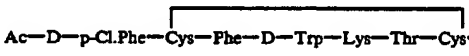
The subsequent amino acid residues are then introduced sequentially by coupling in the same manner as described above.

The deprotection is performed as described for Boc-Thr(Bz)-BHA resin. After incorporating Boc-D-Trp, 5% mercaptoethanol is added to 50% trifluoroacetic acid in CH_2Cl_2; Boc-D-p-Cl.Phe-Cys(MBz)-Phe-DTrp-Lys(2-ClZ)-Thr(Bz)-Cys(MBz)-Thr(Bz)-BHA; resin is obtained. After deprotection and neutralization, acetylation is done by using (Ac_2O) in CH_2Cl_2 for 60 minutes (12.5 m. moles).

Finally, the peptide resin is washed with CH_2Cl_2, methanol and Ch_2Cl_2 three times each and dried under vacuum. 0.902 g. Ac-D-p-Cl.Phe-Cys(MBz)-Phe-D-Trp-Lys(2-ClZ)-Thr(Bz)-Cys(MBz)-Thr(Bz)-BHA resin is obtained.

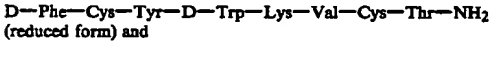
500 mg. protected octapeptide BHA resin is mixed with 0.5 ml. cresol and 0.5 ml. 1,2-ethanedithiol, and stirred in 10 ml. hydrogen fluoride at 0° C. for 1 hour. Excess hydrogen fluoride is evaporated under vacuum. The peptide and resin mixture is washed with ethyl acetate and extracted with 30% HOAc and lyophilized. 112 mg. crude product powder consisting of Ac-D-p-Cl.Phe-Cys-Phe-D-Trp-Lys Thr-Cys-Thr-NH_2 is obtained.

110 mg. crude reduced form is dissolved in 20 ml. 50% HOAc and diluted to 500 ml. with degassed water (N_2), adjusted to pH 6.8 with 28% ammonium hydroxide, then 0.005N potassium ferroxcyanide solution is dropped in with stirring until a permanent yellow color is observed. After stirring for 15 minutes, the pH is readjusted to 5 with HOAc. 5 g. Bio Red AG 3-X 4A resin (chloride form) is introduced to remove ferric and ferrocyanide salts. The filtrate is lyophilized. The residue is subjected to gel filtration on a column (1×120 cm) of Sephadex G15 and eluted with 50% acetic acid. The major peak is lyophilized and 57 mg. of the crude oxidized form,

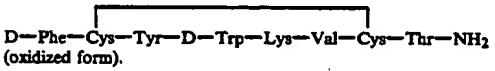


is obtained and the purified by chromatography (high pressure liquid chromatography) to give 10.56 mg. pure octapeptide.

EXAMPLE 2



(reduced form) and

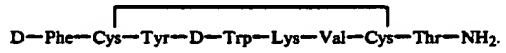


(oxidized form).

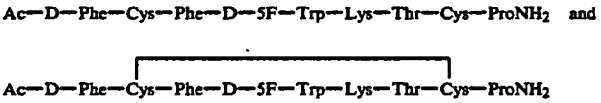
185 mg. BHA resin (0.5 mm BHA/g. resin) is placed in a 20 ml. reaction vessel which is mounted on a mechanical shaker.

The following amino acid residues, Boc-Thr(Bz), Boc-Cys(MBz), Boc-Val, Boc-Lys(2-ClZ), Boc-D-Trp, Boc-Tyr (2-BrZ), Boc-Cys(MBz), and Boc-D-Phe are introduced sequentially by coupling and deprotection in the same manner as described in Example 1. 330 mg. TFA-D-Phe-Cys(MBz)-Tyr(2-BrZ)-D-Trp-Lys(2-ClZ)-Val-Cys(MBz)-Thr(Bz)-BHA resin is finally obtained.

The protected octapeptide amide-resin is treated with HF to give 68.1 mg. crude reduced form. The amount of 6.1 mg. crude reduced form is purified by HPLC to give 1.0 mg. pure D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2. 40 mg. crude form is oxidized as described in Example 1. The lyophilized powder consisting of oxidized form and salts is subjected to gel filtration with Sephadex G15 and eluted with 50% acetic acid. The major peak is then lyophilized to give 38.1 mg. of which 24.7 mg. is purified by HPCL to afford 9.0 mg. of pure



EXAMPLE 3

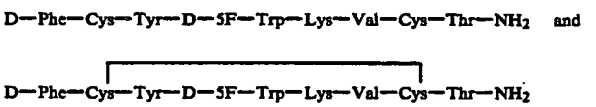


230 mg. benzhydrylamine resin (ca. 0.5 mm BHA/g. resin) is placed in a 5 ml. reaction vessel which is mounted on a mechanical shaker. The protected octapeptide resin is obtained after stepwise coupling of the following:

Boc-Pro, Boc-Cys(MBz), Boc-Thr(Bz), Boc-Lys(2-ClZ), Boc-D/L-5F-Trp, Boc-Phe, Boc-Cys(MBz), Boc-D-Phe.

Acetylation is done after the last deprotection. 351 mg. Ac-D-Phe-Cys(MBz)-Phe-D/L-5F-Trp-Lys(2Clz)-Thr(Bz)-Cys(MBz)-Pro-BHA resin is finally obtained and treated with HF to give 74.5 mg. crude reduced form. The crude peptide in reduced form is oxidized as described in Example 1. After gel filtration, further purification and separation of D/L diastereomers is performed by HPLC.

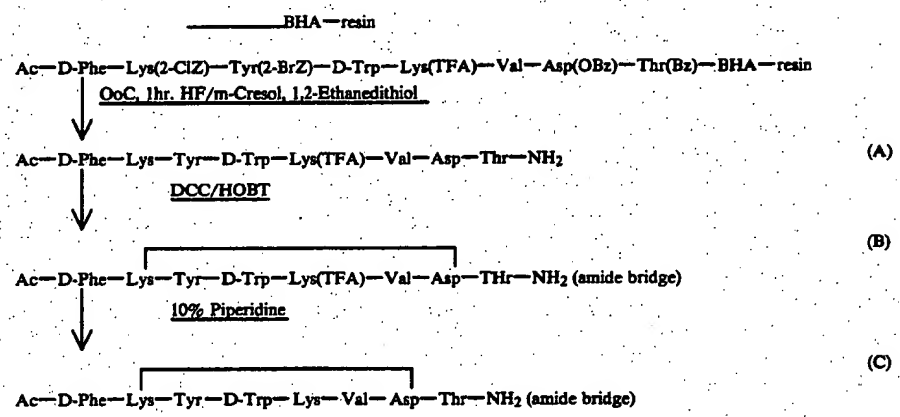
EXAMPLE 4



200 mg. benzhydrylamine resin (ca. 0.36 m. mol. NH_2/g. resin) is placed in a shaking reaction vessel. After addition of the protected amino acids, TFA-D-Phe-Cys(MBz)-Tyr(2-BrZ)-D/L-5F-Trp-Lys(2-ClZ)-

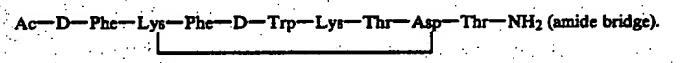
Val-Cys(MBz)-Thr(Bz)-BHA resin (394 mg.) is obtained. The protected resin is treated with HF to give

The synthesis route is described in the following scheme:



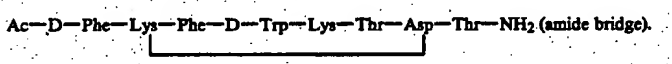
97.5 mg. crude peptide of D-Phe-Cys-Tyr-D/L-5F-Trp-Lys-Val-Cys-Thr-NH_2 (in reduced form). 80 mg. of crude reduced form is oxidized as described in Example 1. After gel filtration, Peaks I and II are lyophilized, yielding 19.8 mg. respectively. Further purification and separation of D/L diastereomers is performed by HPLC.

EXAMPLE 5

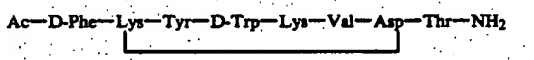


512 mg. Ac-D-Phe-Lys(2-ClZ)-Phe-D-Trp-Lys(TFA)-Thr(Bz)-Asp(OBz)-Thr(Bz)-BHA resin is obtained from 320 mg. benzhydrylamine resin. (TFA=trifluoroacetyl) The protected peptide resin is treated with HF to give 127 mg. crude peptide of Ac-D-Phe-Lys-Phe-D-Trp-Lys(TFA)-Asp-Thr-NH_2. The cyclization procedure is as follows:

100 mg. crude peptide of Ac-D-Phe-Lys-Phe-D-Trp-Lys-Thr(TFA)-Asp-Thr-NH_2 and 50 mg. HOBT are dissolved in 4 ml. DMF and 0.2 m mol. N,N,-diisopropylcarbodiimide in CH_2Cl_2 is added to the solution with stirring at 0° C. for 2 hours and at room temperature for 5 hours until a negative ninhydrin test is obtained. The solvent is evaporated under vacuum. Removal of TFA-group of Lys in the peptide is performed in a solution of piperidine:DMF:H_2O=10:45:45 by stirring at room temperature for 3 hours. The DMF and piperidine are removed by evaporation. The oily material is subjected gel filtration on Sephadex G15 (1×120 cm). The peaks I and II are lyophilized to give 30 mg. and 48.4 mg., respectively. The peak II is purified by HPLC to give 25 mg. pure peptide of



EXAMPLE 6

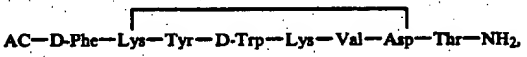


Procedure:

2.14 g. Ac-D-Phe-Lys(2-ClZ)-Tyr(2-BrZ)-D-Trp-Lys(TFA)-Val-Asp(OBz)-Thr(Bz)-BHA-resin is obtained from 1.5 g. benzhydrylamine resin (0.26 m. mol/g.). The protected peptide resin is treated with HF to give 279 mg. crude peptide of Ac-D-Phe-Lys-Tyr-D-Trp-Lys(TFA)-Val-Asp-Thr-NH_2 (A).

40 mg. crude peptide (A) is purified by HPLC. The rest of crude peptide (A) is cyclized with diisopropylcarbodiimide and HOBT using the same procedure as described in Example 5.

One sixth of the reaction mixture of Ac-D-Phe-Lys-Tyr-D-Trp-Lys(TFA)-Val-Asp-Thr-NH_2 (B) is subjected to gel filtration on Sephadex G15 (1×120 cm) and eluted by 30% acetic acid. HPLC is used as a guide to combine the peaks separately. The peptide eluted is lyophilized and purified by HPLC to give pure peptide (B). The rest of the reaction mixture is dissolved in a solution of piperidine:DMF:H_2O=10:45:45 and stirred at room temperature for 3 hours. The solvent is evaporated under vacuum. The oily material is subjected to gel filtration on Sephadex G15 (1×120 cm) and eluted with 30% acetic acid. The peaks I and II are lyophilized to give 58.3 mg. and 127.3 mg. respectively. Further purification of peak II is performed by HPLC to give 80 mg. pure peptide (C),



(amide bridge).

EXAMPLE 7

In a similar manner, utilizing the techniques and procedures described in Examples 1–6, there are obtained the following compounds of this invention:

TABLE 1

Formula (I) and (I')
A.Cys.Phe.D-Trp.Lys.Thr.Cys.B (I) (reduced form)

A.Cys.Phe.D-Trp.Lys.Thr.Cys.B (I') (oxidized form)

A	B	Formula
DAla	Thr NH_2	I
DVal	"	"
Ac.Phe	"	"
D-Phe	"	"
Dphe	"	I'
Ac.D-Phe	"	I
p.Cl.D-Phe	"	"
p.Cl.Dphe	"	I'
Ac.p.Cl.D-Phe	"	I
Ac.p.Cl.D-Phe	"	I'
Ac.Trp	"	I
Ac.Trp(For)	"	"
Ac.D-Trp	"	"
D-Trp	"	"
Ac.Pro	"	"
D.Pro	"	"
Ac.Pro(OH)	"	"
Ac.Ser	"	"
D.Ser	"	"
Ac.Thr	"	"
D-Thr	"	"
Ac.Tyr	"	"
Dp-Glu	"	"
Ac.D-Phe	Val NH_2	"
Ac.D-Phe	Pro NH_2	"
D.Ser	(HO)Pro NH_2	"
D-Phe	(HO)Pro NH_2	"
"	Ser NH_2	"
D.Ser	"	"
D-Phe	D.Ser NH_2	"
D-Phe	Tyr NH_2	"
Ac.D-Phe	Trp NH_2	"
"	D-Trp NH_2	"
"	D/L 5F Trp NH_2	"
Ac.Trp(For)	For | Trp NH_2	"
"	D/L 5F Trp NH_2	"
Ac.D.Trp	D.Trp NH_2	"
"	For | Trp NH_2	"
Ac.Trp	D-Trp NH_2	"
Ac.Trp(For)	Pro NH_2	"
p.Cl D-Phe	"	"
Ac.Pro	"	"
D.Ser	"	"
Ac.Pro	For | Trp NH_2	"
Ac.D-Phe	"	"
Ac D/L 5F Trp	Thr NH_2	"

TABLE 2

Formula (II) and (II')
A.Cys.Tyr.D—Trp.Lys.Val.Cys.B (II)

A.Cys.Tyr.D—Trp.Lys.Val.Cys.B (II')

A	B	Formula
Ac Gly	Thr NH_2	II
D.Val	"	"
Ac.Phe	"	"
D—Phe	"	"
D—Phe	"	II'
Ac—Dphe	"	II

TABLE 2-continued

Formula (II) and (II')
A.Cys.Tyr.D—Trp.Lys.Val.Cys.B (II)

A.Cys.Tyr.D—Trp.Lys.Val.Cys.B (II')

A	B	Formula
Ac—Dphe	"	II'
p.Cl.D—Phe	"	II
"	"	II'
Ac.Pro.	"	II
D.Pro	"	"
Dp—Glu	"	"
D—Glu	"	"
D—Trp	"	"
Ac.Ser	"	"
D.Ser	"	"
D.Tyr	"	"
D.Ala.Phe	"	"
D—Phe	Thr Ala NH_2	"
"	Ala NH_2	"
"	Gly NH_2	"
"	Me Ala NH_2	"
"	Abu—NH_2	"
p.Cl.D—Phe	Ala NH_2	"
D—phe	Pro NH_2	"
"	DPro NH_2	"
"	(Ho)Pro NH_2	"
Ac—LPhe	"	"
D—Phe	Tyr NH_2	"
"	Ser NH_2	"
"	"	II'
D.Ser	Ala NH_2	"
"	(Ho)Pro NH_2	"
"	Ser NH_2	"
"	Tyr NH_2	"
DVal	Ala NH_2 For	"
D—Phe	Trp NH_2	"
Dphe	"	II'
p.Cl D—Phe	"	II
DVal	"	"
Ac—Pro	"	"
Ac Trp(For)	D/L 5F Trp NH_2	"
D—Phe	"	"
"	"	II'
"	TrpNH$_2$	II
"	"	II'

TABLE 3

Formula (III) and (IV')
A.Cys.Phe.Z.Lys.Thr.Cys.B (III)

A.Cys.Phe.Z.Lys.Thr.Cys.B (III')

A.Cys.Tyr.Z.Lys.Val.Cys.B (IV)

A.Cys.Tyr.Z.Lys.Val.Cys.B (IV')

A	B	Z	Formula
Ac.p.Cl.D—Phe	Thr NH_2	D/L 5F Trp	(III)
Ac.D—Phe	Pro NH_2	D.Trp	"
"	"	Trp For	"
"	"	Trp	"
"	"	D/L 5F Trp	"
Ac.Dphe	"	D/L 5F Trp	(III')
D—Phe	"	"	(III)
Ac.p.Cl.D—Phe	"	"	"
D—Phe	(Ho)Pro NH_2	"	"
D.Ser	"	"	"
p.Cl.D—Phe	Thr NH_2	"	(IV)
D—Phe	"	"	(IV)
"	"	"	(IV')
"	"	Trp	(IV)

TABLE 3-continued

Formula (III) and (IV')

A.Cys.Phe.Z.Lys.Thr.Cys.B (III)

A.Cys.Phe.Z.Lys.Thr.Cys.B (III')

A.Cys.Tyr.Z.Lys.Val.Cys.B (IV)

A.Cys.Tyr.Z.Lys.Val.Cys.B (IV')

A	B	Z	Formula
"	"	"	(IV')

TABLE 4

Formula (V) and (VI)

A.C.phe.D—Trp.Lys.Thr.C'.B (V)

A.C.Tyr.D—Trp.Lys.Val.C'.B (VI)

A	B	C	C'	Formula
D—Phe	Thr NH_2	Cys	Cys	(V)
"	"	D Cys	D Cys	"
"	"	D Cys	Cys	"
"	"	Cys	D Cys	"
Ac'—DPhe	"	Lys	Asp (amide br)	"
Ac'—D—Phe	"	"	"	(VI)

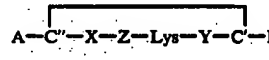
TABLE 5

Calculation of Gastric and Inhibitory Activity of Octapeptide
RC—88-II = p-Cl—D-Phe—Cys—Tyr—D-Trp—Lys—Val—Cys—Thr—NH_2 on the basis of 16 Experiments in Dogs with gastric Fistulae.

RAW DATA STANDARD DOSE LOW	HIGH	TESTED MATERIAL DOSE LOW	HIGH		
3	6	3	6		
49	23.6	22.5	12		
48	25.8	23	9		
71	35.9	35.6	27		
53	27	22.8	16		
		SUMS OF SQUARES	DF	MEAN SQUARE	F RATIO
ROW (MATERIAL)		1709.82	1	1709.82	27.6527
COLUMN (DOSE)		1380.12	1	1380.12	22.3205
INTERACTION		295.839	1	295.839	4.78456
SUBTOTAL		3385.79	3	1128.6	
WITHIN DOSES		741.984	12	61.185	
TOTAL		4127.77	15	275.185	
SDT ERROR:	7.86333				
	F. VALUES				
ROW (SAMPLE)		27.6527	SIGNIFICANT		
COLUMN (SLOPE)		22.3205	SIGNIFICANT		
INTERACTION (PARALLEL)		4.78458	SIGNIFICANT		
SAMPLE POTENCY = 216.303%					
POTENCY RANGE FROM		140.669%	TO	332.605%	
LAMBDA (DEGREES OF PRECISION) = .234861E-01					

We claim:

1. A compound of the formula



(I)

wherein

A is an L, D or DL amino acid selected from the group consisting of phenylalanine (Phe), its acetylated derivatives or a pharmaceutically acceptable acid addition salt thereof;

B is an L, D or DL amino acid selected from the group consisting of threonine amid (Thr NH_2), tyrosine amide (Tyr NH_2), tryptophan amide (Trp NH_2);

X is L-phenylalanine (L-Phe) or L-tyrosine (L-Tyr);

Y is L-valine (L-Val);

Z is D-tryptophan (D-Trp); and

C'' and C' are L or D-cysteine (Cys), -aminobutyric acid (Abu), aspartic acid (Asp) or lysine (Lys);

provided that where C' is Cys, C'' is also Cys and where C' or C'' are other than Cys, C'' is different from C' and is other than Cys;

the connecting line between C'' and C' signifies a bridge selected from the group consisting of carbon/carbon, carbon/sulfur, sulfur/sulfur and amide bridges; and the pharmaceutically acceptable acid addition salts thereof.

2. An octapeptide (reduced form) of the formula

A-C''-X-Z-Lys-Y-C'-B

wherein A, C'', X, Z, Y, C' and B are as defined in claim 1.

3. A compound according to claim 1 wherein

C'' is Cys;

X is Tyr;

Z is D-Trp;

Y is Val; and

C' is Cys.

4. A compound according to claim 1 wherein

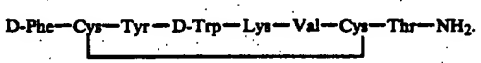
C'' is Cys;

X is Tyr;

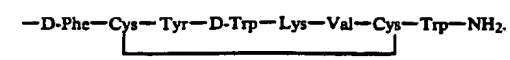
Y is Val; and

C' is Cys.

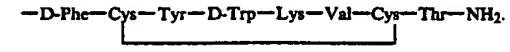
5. A compound according to claim 1 which is



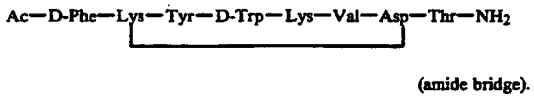
6. A compound according to claim 1 which is



7. A compound according to claim 1 which is



8. A compound according to claim 1 which is



(amide bridge).

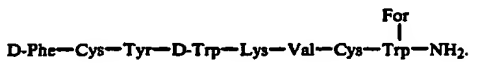
9. An octapeptide (reduced form) of the formula

wherein A, C'', X, Z, Y, C' and B are as defined in claim 1.

10. A compound according to claim 2 which is: D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2.

11. A compound according to claim 9 which is: D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Ser-NH_2.

12. A compound according to claim 9 which is:



13. A compound according to claim 12 which is: D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Tyr-NH_2.

14. A pharmaceutical composition effective for reducing growth hormone serum levels which comprises an octapeptide of claim 1, its reduced form or a pharmaceutically acceptable acid addition salt thereof in a pharmaceutically acceptable liquid or solid carrier thereof.

15. A pharmaceutical composition of claim 14 which is encapsulated in poly(d,l-lactide-co-glycolide) microcapsules.

16. A method of treating excess release of growth hormone, gastrointestinal disorders, and diabetes in a mammal in need of such therapy which comprises administering to said mammal an effective dose of octapeptide of claim 1, its reduced form, or a pharmaceutically acceptable acid addition salt thereof.

* * * * *

EXHIBIT 2

Authorization for H3 Pharma to Apply for Extension of Patent Term

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No. : 4,650,787
Patentee : Schally et al.
Issue Date : March 17, 1987
Serial No. : 727,150
Filed : April 25, 1985
Title : BIOLOGICALLY ACTIVE OCTAPEPTIDES

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

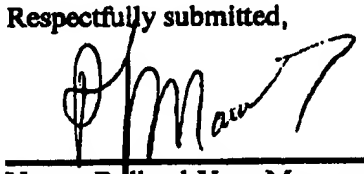
AUTHORIZATION FOR H3 PHARMA TO APPLY FOR EXTENSION OF PATENT TERM

Pursuant to 37 C.F.R. § 1.730, Debiopharm S.A. and Debio Recherche Pharmaceutique S.A. (collectively "Debio") hereby appoint H3 Pharma, Inc. ("H3 Pharma"), a Canadian corporation, as Debio's sole agent for the purposes of pursuing interim and permanent patent term extensions for United States Patent No. 4,650,787 ("the '787 patent"), as provided under 35 U.S.C. § 156. H3 Pharma will do all things useful or necessary in order to seek and obtain such patent term extensions for the '787 patent. Debio will provide H3 Pharma with all information requested by H3 Pharma for purposes of pursuing such patent term extensions for the '787 patent.

Debio certifies that it is the exclusive licensee of the '787 patent, by virtue of a license received from The Administrators of The Tulane Educational Fund ("Tulane"), the assignee of 100% of the right, title and interest in the '787 patent. The undersigned has reviewed this license, and to the best of undersigned's knowledge and belief, Tulane has exclusively granted all patent rights in the '787 patent to Debio, including the right to pursue patent term extensions. The undersigned, whose title is supplied below, is empowered to act on behalf of Debio.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,



Date: March 16, 2005

Name: Rolland-Yves Mauvernay
Title: President and CEO of
Debiopharm S.A.
and of
Debio Recherche Pharmaceutique S.A.

EXHIBIT 3

Assignment from Andrew V. Schally and Renzhi Z. Cai to Tulane

Patent Assignment Abstract of Title

NOTE:Results display only for issued patents and published applications. For pending or abandoned applications please consult USPTO staff.

Total Assignments: 1

Patent #: 4650787 **Issue Dt:** 03/17/1987 **Application #:** 06727105 **Filing Dt:** 04/25/1985

Inventors: ANDREW V. SCHALLY, REN Z. CAI

Title: BIOLOGICALLY ACTIVE OCTAPEPTIDES

Assignment: 1

Reel/Frame: 004726/0891 **Recorded:** 06/15/1987 **Pages:** 2

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST.

Assignors: SCHALLY, ANDREW V. **Exec Dt:** 05/12/1987

CAI, REN Z. **Exec Dt:** 05/12/1987

Assignee: ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND OF NEW ORLEANS, LOUISIANA, THE,

Correspondent: OMRI M. BEHR
325 PIERSON AVENUE
EDISON, NJ 08837

Search Results as of: 2/12/2004 11:59:18 A.M.

If you have any comments or questions concerning the data displayed, contact OPR / Assignments at 703-308-9723
Web interface last modified: Oct. 5, 2002

Assignment, Sole/Joint
Concurrent/pre SN Exec.

FILE NO: KIRK 3.0-001

ASSIGNMENT

WHEREAS, We, Andrew V. Schally and ~~Ren~~ RENZHI Z. Cai ---------

as assignor , have invented certain improvements in Biologically active octapeptides ----------

for which ---------- United States Letters Patent 4,650,787 were granted on March 17th 1987----------
and whereas

The Administrators of the Tulane Educational Fund of New Orleans, Louisiana ----------

as assignee, is desirous of acquiring all right, title and interest in and to said invention and any Letters Patent that may be granted therefor.

NOW, THEREFORE, in consideration of One Dollar ($1.00) and other good and valuable consideration, the receipt of which is hereby acknowledged, we , as assignors hereby sell, assign and set over to said assignee the entire right, title andinterest for the United States and all other countries in and to said invention and the aforesaid ---------- Letters Patent, all original, divisional, continuation, substitute or reissue applications and patents applied for or granted therefor in the United States and all other countries and the Commissioner of Patents is hereby authorized and requested to issue all patents on said improvements or resulting therefrom to said assignee herein, as assignee of the entire interest therein; and the undersigned for us and our legal representatives, heirs and assigns do hereby agree and covenant without further remuneration, to execute and deliver all divisional, continuation, reissue and other applications for Letters Patent on said improvements and all assignments thereof to said assignee or its assigns, to communicate to said assignee or its representatives all facts known to the undersigned respecting said improvements, whenever requested, to testify in any interferences or other legal proceedings in which any of said applications or patents may become involved to sign all lawful papers, make all rightful oaths, and to do generally everything necessary to aid assignee, its successors, assigns and nominees to obtain patent protection for said improvements in all countries, the expenses incident to said applications to be borne and paid by said assignee.

Andrew V. Schally

Andrew V. Schally

Renzhi Cai

~~Ren~~ RENZHI Z. Cai

STATE OF Louisiana)
COUNTY OF ORLEANS PARISH)SS.:

This 12th day of May ---------- 1987
before me personally came the above named Andrew V. Schally ----------
to me personally known as the individual who executed the foregoing instrument who acknowledged to me that the same was executed by of own free will for the purposes therein set forth.

NOTARY PUBLIC
SEAL

The application for Letters Patent identified in this assignment having been executed on 4/18/87 has, by the U.S. Patent Office, been given Serial No. 07/053,101 and filing date of 4-27-87

REEL 4726 FRM 0891

STATE OF LOUISIANA)
COUNTY OF ORLEANS PARISH)SS.:

This 12th day of May, 1987,
before me personally came the above named ~~Ren Z~~. Cai Renzhi
to me personally known as the individual who executed the foregoing instrument who acknowledged to me that the same was executed by of own free will for the purposes therein set forth.

NOTARY PUBLIC
SEAL

RECORDED
PATENT & TRADEMARK OFFICE
JUN 15 1987
COMMISSIONER OF PATENTS AND TRADEMARKS OFFICE

REEL 4728 FRAME 894

EXHIBIT 4

Structural Formula of Vapreotide

Exhibit 4: Structural Formula of Vapreotide

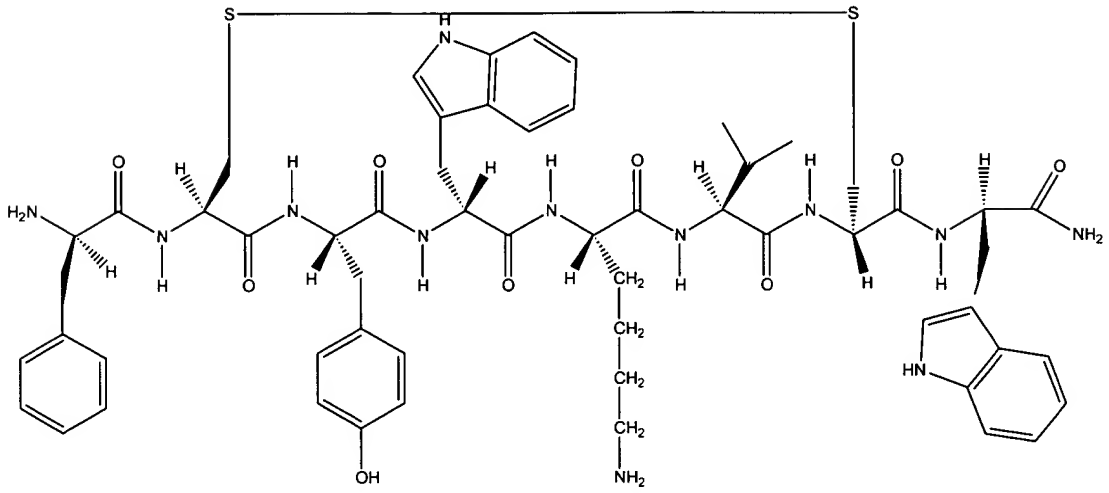


EXHIBIT 5

Steps for Synthesis of Vapreotide Acetate

Exhibit 5: Steps for Synthesis of Vapreotide Acetate

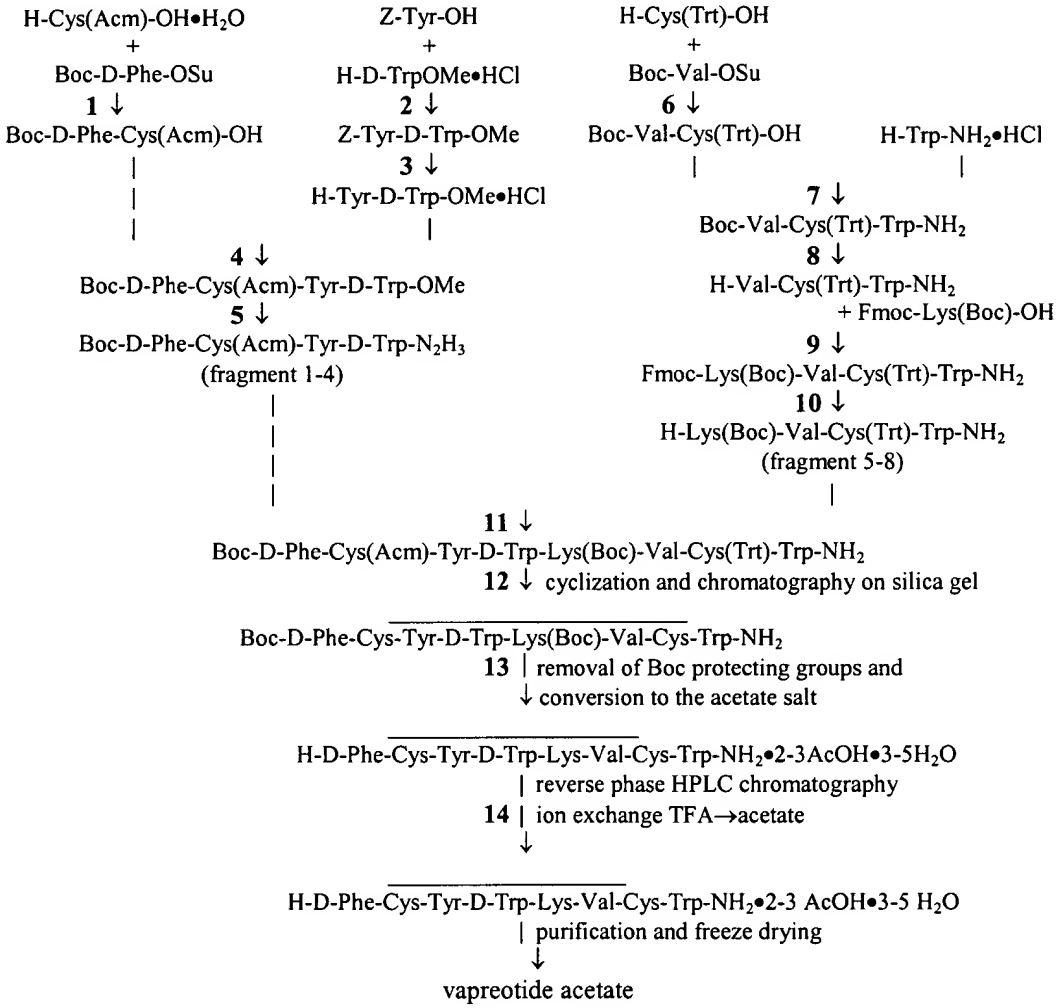


EXHIBIT 6

Maintenance Fee Statement for United States Patent Number 4,650,787

Patent Maintenance Fees 02/12/2004

Patent Number	4650787	**Issued**	03/17/1987	**Application Number**	06727105	**Filed**	04/25/1985
Status	4th, 8th and 12th year fees paid					**Small Entity**	NO
Window Opens		**Surchg Due**				**Expiration**	
Fee Amt Due		**Surchg Amt Due**				**Total Amt Due**	
Fee Code							
Surchg Code							
Title	BIOLOGICALLY ACTIVE OCTAPEPTIDES						

Address for Fee Purpose

OMRI M. BEHR
325 PIERSON AVENUE
EDISON NJ 08837 US

Most Recent Significant Events (up to 7)

1998/08/20 Pat Hldr no Longer Claims Small Ent Stat as Indiv Inventor.
1998/08/17 Payment of Maintenance Fee, 12th Year, Large Entity.
1994/06/28 Payor Number Assigned.
1994/06/28 Payer Number De-assigned.
1994/06/17 Payment of Maintenance Fee, 8th Yr, Small Entity.
1990/09/26 Payor Number Assigned.
1990/09/10 Payment of Maintenance Fee, 4th Yr, Small Entity, PL 97-247.
End of Maintenance History

New Query Print

EXHIBIT 7

Approvable Letter for Sanvar

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

NDA 21-761

J. Kay Noel & Associates
Attention: Kay Noel, Ph.D.
U.S. Agent for H3 Pharma, Inc.
8371 Terrace Drive
El Cerrito, CA 94530

Dear Dr. Noel:

Please refer to your new drug application (NDA) dated February 27, 2004, received March 1, 2004, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Sanvar™ (vapreotide) Injection, 0.6 mg.

We acknowledge receipt of your submissions dated May 13, June 30, July 12 and 22, August 3 and 13, September 9 and 22, October 7 and 8, and November 1, 2004.

We completed our review of this application, as amended, and it is approvable. Before the application may be approved, however, it will be necessary for you to address the following:

Clinical

There is lack of substantial evidence that demonstrates the efficacy of vapreotide for the use in treatment of acute variceal bleeding related to portal hypertension associated with endoscopic treatment. Provide additional efficacy data from a well-controlled clinical trial. We understand you have just completed a major trial.

Chemistry, Manufacturing and Quality Control

1. Develop a stability-indicating assay that will be used for vapreotide release and stability testing. The proposed assay procedure is not selective for vapreotide.

2. The assay procedure for related impurities must unambiguously differentiate between cyclic vapreotide and the linear octapeptide analog.

3. The deficiencies outlined in the December 2, 2004, Agency letter must be addressed.

4. With respect to the drug product stability assay and impurities testing procedures, provide evidence that the methods are selective for vapreotide and related substances, and that the methods are stability indicating and suitable for quantification. If the methods cannot be supported with

NDA 21-761
Page 2

suitable evidence, then new more suitable methods must be developed and new stability studies conducted with the new methods.

When you respond to the above deficiencies, include a safety update as described at 21 CFR 314.50(d)(5)(vi)(b). The safety update should include data from all non-clinical and clinical studies of the drug under consideration regardless of indication, dosage form, or dose level.

1. Describe in detail any significant changes or findings in the safety profile.

2. When assembling the sections describing discontinuations due to adverse events, serious adverse events, and common adverse events, incorporate new safety data as follows:

   - Present new safety data from the studies for the proposed indication using the same format as the original NDA submission.
   - Present tabulations of the new safety data combined with the original NDA data.
   - Include tables that compare frequencies of adverse events in the original NDA with the retabulated frequencies described in the bullet above.
   - For indications other than the proposed indication, provide separate tables for the frequencies of adverse events occurring in clinical trials.

3. Present a retabulation of the reasons for premature study discontinuation by incorporating the drop-outs from the newly completed studies. Describe any new trends or patterns identified.

4. Provide case report forms and narrative summaries for each patient who died during a clinical study or who did not complete a study because of an adverse event. In addition, provide narrative summaries for serious adverse events.

5. Describe any information that suggests a substantial change in the incidence of common, but less serious, adverse events between the new data and the original NDA data.

6. Provide a summary of worldwide experience on the safety of this drug. Include an updated estimate of use for drug marketed in other countries.

7. Provide English translations of current approved foreign labeling not previously submitted.

In addition, we have identified the following issue not related to approvability. Your application does not contain information about vapreotide's effect on QT. Provide analysis of QT prolongation potential by vapreotide from available sources including published literature, drug history including other members of the class, pre-clinical data, Phase 3 data, and EKGs from INDs pertinent to the current indication or other indications being studied.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Florence Houn
12/21/04 11:52:08 AM

EXHIBIT 8

Brief Description of Significant Activities Undertaken During Regulatory Review Period

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
1	August 26, 1999	Letter	J. Kay Noel J. Kay Noel & Associates	Office of Orphan Products Development	Orphan drug Application	Request for orphan drug designation for vapreotide acetate for prevention of postoperative complications following pancreatic resection, treatment of esophageal variceal hemorrhage, gastrointestinal and pancreatic fistulas
2	November 5, 1999 (received November 23, 1999)	Letter	J. Kay Noel J. Kay Noel & Associates	Center for Drug Evaluation and Research	Investigational new drug application	Submission of investigational new drug application for phase III clinical trials of vapreotide acetate.
3	December 1, 1999	Letter	Center for Drug Evaluation and Research	J. Kay Noel J. Kay Noel & Associates	Investigational new drug application	Acknowledgement of receipt of investigational new drug application for vapreotide acetate.
4	February 2, 2000	Letter	J. Kay Noel J. Kay Noel & Associates	Paul E. Levine FDA	Submission of additional nonclinical study information	Submission of additional nonclinical study information regarding IND 59,287 for vapreotide acetate.
5	May 1, 2000	Letter	J. Kay Noel J. Kay Noel & Associates	Paul E. Levine FDA	Request for pre-NDA meeting	Request for Pre-NDA meeting for vapreotide acetate for the treatment of esophageal variceal hemorrhage
6	June 28, 2000	Letter	J. Kay Noel J. Kay Noel & Associates	Paul E. Levine FDA	Response to FDA review of study protocol DEB-98-VAP-06	Further to letter dated 11 April, 2000, where 3 suggestions for revision of the Phase III study were proposed, replies from Steering Committee and Sponsor, re these suggestions.

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
7	June 28, 2000	Letter	J. Kay Noel J. Kay Noel & Associates	Kamel Besseghir Debiopharm	Minutes of pre-NDA meeting with FDA	Minutes of pre-NDA meeting with FDA for the treatment of esophageal variceal hemorrhage
8	July 11, 2000	Letter	Center for Drug Evaluation and Research	J. Kay Noel J. Kay Noel & Associates	Investigational new drug application	FDA completed phase III protocol review for investigational new drug application for vapreotide acetate
9	August 25, 2000	Letter	J. Kay Noel J. Kay Noel & Associates	Paul E. Levine FDA	Response to minutes of pre-NDA meeting with FDA	Response to minutes of pre-NDA meeting with FDA for the treatment of esophageal variceal hemorrhage
10	September 18, 2000	Letter	J. Kay Noel J. Kay Noel & Associates	Paul E. Levine FDA	Preclinical protocol outline for review	Preclinical protocol outline for review for 28-day continuous IV infusion study in rats and dog followed by a 14-day recovery period.
11	November 16, 2000	Letter	J. Kay Noel J. Kay Noel & Associates	Alice Kacuba FDA	Questions regarding preclinical study protocol	Questions regarding preclinical study protocol for 28-day continuous IV infusion study in rats and dogs followed by a 14-day recovery period
12	March 9, 2001	Letter	J. Kay Noel J. Kay Noel & Associates	Alice Kacuba FDA	Statistical analysis plan	Statistical analysis plan for the planned interim analysis of study DSB-98 VAP-06
13	March 26, 2001	Letter	J. Kay Noel J. Kay Noel & Associates	Marlene E. Haffner FDA	Notification of early termination of study	Notification of early termination of study DEB-98-VAP-06

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
14	October 29, 2002	Letter	J. Kay Noel J. Kay Noel & Associates	Marlene E. Haffner FDA	Annual progress report re Orphan Drug Application #99-1298	Further to meeting on June 20, 2000 wherein FDA reviewers noted that Debiopharm would need to address 3 major deficiencies in order to file an NDA for Octastatin for the treatment of esophageal variceal hemorrhage in cirrhotic patients. Summary of steps taken since then.
15	March 25, 2003	E-mail			Response to issues raised in pre-NDA meeting	
16	April 25, 2003	Letter	J. Kay Noel J. Kay Noel & Associates	Alice Kacuba FDA	Response to issues raised in FDA meeting held June 20, 2000	Response to issues raised in FDA meeting held June 20, 2000 to discuss a proposed NDA for Octastatin for the treatment of acute variceal hemorrhage in cirrhotic patients.
17	May 16, 2003	Letter	J. Kay Noel J. Kay Noel & Associates	Tanya D. Clayton FDA	Request for pre-NDA meeting	Completed form FDA 1571 and request for type B meeting to discuss an NDA for Octastatin injection for the treatment of variceal hemorrhage in cirrhotic patients. Asking them to suggest meeting dates towards end of statutory 60 days from Agency receipt of written request for a meeting
18	July 11, 2003	Fax	Tanya D. Clayton FDA	J. Kay Noel J. Kay Noel & Associates	Response to questions in meeting request	Responses to questions listed in JKN June 13 background package.

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
19	August 5, 2003	Letter	J. Kay Noel J. Kay Noel & Associates	Tanya D. Clayton FDA	Request for waiver	Request for waiver of evidence of in vivo bioequivalence for vapreotide acetate
20	August 5, 2003	Letter	J. Kay Noel J. Kay Noel & Associates	Tanya D. Clayton FDA	Request for waiver	Request to waive requirements of carcinogenicity testing for vapreotide acetate.
21	August 5, 2003	Letter	J. Kay Noel J. Kay Noel & Associates	Tanya D. Clayton FDA	Request for waiver	Request to waive requirements for male fertility and peri-postnatal reproduction toxicity studies for vapreotide acetate
22	August 5, 2003	Letter	J. Kay Noel J. Kay Noel & Associates	Tanya D. Clayton FDA	Request for waiver	Request to waive requirements to comply with a pediatric rule for vapreotide acetate
23	February 27, 2004	Fax	J. Kay Noel J. Kay Noel & Associates	Didier Coquoz H3 Pharma		
24	April 5, 2004	Fax	Tanya Clayton FDA	J. Kay Noel J. Kay Noel & Associates	Request for additional copies of Modules 1 & 2	3 additional desk copies of Modules 1 & 2 to be labeled 'Desk Copy'
25	April 6, 2004	Fax	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Provision of additional copies	Further to April 5, 2004 request, 3 additional desk copies of Modules 1 & 2 forwarded. Total submission = 9 volumes
26	April 7, 2004	Fax	Tanya Clayton FDA	J. Kay Noel J. Kay Noel & Associates	Request for location of information in NDA	For the survival in the per protocol population for both studies

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
27	April 8, 2004	Fax	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Response to April 7 fax	Study DEB-96-VAP-14 (French) and Study DEB-01-VAP-07 (Egyptian)
28	April 13, 2004	Fax	J. Kay Noel J. Kay Noel & Associates	Robert Vinson H3 Pharma	Orphan drug designation	Fax advising receipt of notification letter (dated April 6, 2004) from FDA regarding grant of orphan drug designation for vapreotide for the treatment of symptomatic carcinoid tumors.
29	April 20, 2004	Fax	Tanya Clayton FDA	J. Kay Noel J. Kay Noel & Associates	Request for SAS files	For 2 studies DEB-96-VAP-14 and DEB-01-VAP-07 in E-format.
30	April 29, 2004	e-mail	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Response to April 20 fax	Advising that transfer files for data for 2 studies should be available by week of May 10, 2004
31	April 29, 2004	Letter	Tanya Clayton FDA	J. Kay Noel J. Kay Noel & Associates	Acknowledgement of receipt of NDA	Date of receipt: March 1, 2004, Reference number: NDA 21-761
32	May 13, 2004	Letter	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Provision of SAS data sets re April 20 Fax	Further to information request from statistical reviewer (April 20, 2004) , transfer files for data for 2 studies are enclosed.
33	May 14, 2004	Letter	Brian K. Strongin FDA	J. Kay Noel J. Kay Noel & Associates	Acceptance of NDA for review	Filing Couumnication further to NDA submitted 27 February, 2004, FDA has determined that our application is sufficiently complete to permit a substantive review.

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
34	June 25, 2004	Fax	J. Kay Noel J. Kay Noel & Associates	Laura King H3 Pharma	Request for efficacy analysis of DEB-97-VAP-02	Also set up a teleconference with the statistician who prepared the SAS transfer files and performed the Statistical analysis for studies DEB-94-VAP-14 and DEB-01-VAP-07
35	June 28, 2004	e-mail	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Submission of new SAS directory	New SAS directory received today from Debiopharm for SAS transfer files for pivotal studies DEB-96-VAP-14 and DEB-01-VAP-07
36	June 29, 2004	E-Mail	J. Kay Noel J. Kay Noel & Associates		Teleconference held with statistical reviewer	Minutes of Teleconference of 29 June 2004. FDA, H3, Debio, K Noel
37	June 30, 2004	e-mail	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Advising T. Clayton of transmission of files	Advising that due to the size of attachments, (that did not go through), files were forwarded via overnight courier .
38	July 1, 2004	Fax	J. Kay Noel J. Kay Noel & Associates	Laura King H3 Pharma	Request for translations of 2 study reports	Requesting for English translations of full study reports for preclinical studies T955 and 950446T to be provided to FDA
39	July 12, 2004	Fax	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Advised FDA of ongoing study DEB-02-VAP-06	H3 advised that Debiopharm is conducting another Phase III study of Sanvar injection for treatment of acute variceal bleeding related to portal hypertension, in association with endoscopic treatment, using a study design indentical to the pivotal study (DEB-96-VAP-14) submitted in the NDA.

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
40	July 12, 2004	Fax	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Submission of SAS data sets re June 29 telecon	Additional requested data in SAS transfer files supplied further to 29 June, 2004 teleconference.
41	July 16, 2004	e-mail	J. Kay Noel J. Kay Noel & Associates	Laura King H3 Pharma	Info requested by FDA re site audits	Identifying 3 sites to be inspected as well as information yet to be provided to FDA prior to July 21.
42	July 22, 2004	Letter			Submission of final PK report H3PVAP09	
43	July 26, 2004	E-Mail	Centre Hospitalier Universitaire d'Angers	Dr, Malek FDA	re upcoming FDA inspection	Letter confirming that FDA will have full access during audit visit to available patient records related to protocol, be allowed to make copies of parts of the records, be provided with adequate work space for the duration of the audit
44	August 2, 2004	E-Mail			Reply regarding sites to be inspected	
45	August 2, 2004	E-Mail	Khairy Malek FDA	J. Kay Noel J. Kay Noel & Associates	FDA called K. Noel re inspection of M-Scan	Asking location of 3rd reunion site. Advised that inspection will most probably be at 2 sites only.
46	August 5, 2004	E-Mail	Linda Adams FDA	Bernhard Eggimann H3 Pharma	Request from field inspector –copy CMC vols.	Trying to plan inspection at Genzyme on Sept 15-17 and requests the CMC section to be sent to the investigator.
47	August 6, 2004	E-mail	J. Kay Noel J. Kay Noel & Associates	Bernhard Eggimann H3 Pharma	K. Noel spoke to field inspector re sending vol's	Reminder that field copy of NDA plus all CMC volumes plus Modules 1 & 2 must be submitted.

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
48	August 9, 2004	E-mail	J. Kay Noel J. Kay Noel & Associates	Robert Vinson H3 Pharma	Request from stats review for race and PT numbers	Advising that written request recvd from statistical reviewer asking of race data for NDA-761 but that this info was not collected in CRF's for these studies.
49	August 10, 2004	Letter	Maria Iacovelli Genzyme Corp.	Bernhard Eggimann H3 Pharma	Notice from Genzyme re FDA inspection	Letter advising upcoming FDA inspection to take place 15 and 17, September, 2004 to ensure that Vapreotide acetate is manufactured according to current GMP's
50	August 10, 2004	Fax	J. Kay Noel J. Kay Noel & Associates	Bernhard Eggimann H3 Pharma	FDA CMC questions	Information request letter from FDA re: CMC information
51	August 13, 2004	E-mail	J. Kay Noel J. Kay Noel & Associates	H3 Pharma	Request for status update from FDA	Message advising that FDA supervisor was inquiring regarding expected response to four outstanding information requests for NDA-21-761. Advising that reply was needed early next week.
52	August 13, 2004	Fax	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Reply to request for race info VAP-14, VAP-07	
53	August 22, 2004	E-Mail	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Outstanding requests update to T. Clayton	Updates on requests of June 25, July 1 and July 26, 2004.

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
54	September 9, 2004	E-Mail	J. Kay Noel J. Kay Noel & Associates	Tanya Clayton FDA	Submission of preclinical reports (Jul/01) (Translated)	English language translation of study reports as follows: Study No. T955 (TRISA) and Study No. 950446T (CERB).
55	September 13, 2004	Fax	Diane Moore FDA	J. Kay Noel J. Kay Noel & Associates	Request for electronic data set VAP-14	Request to provide data for pivotal study DEB-96-VAP-14 including specific requests for info.
56	September 15, 2004	Fax	Diane Moore FDA	J. Kay Noel J. Kay Noel & Associates	Request for clinical data for VAP-14 and VAP-07	Request for specific information in order to continue with the evaluation of NDA-21-761
57	September 19, 2004	E-Mail	J. Kay Noel J. Kay Noel & Associates	Laura King H3 Pharma	Clinical Data for VAP-14 and VAP-07	Message asking for selected clinical info on DEB-96-VAP-14 and DEB-01-VAP-07 as well as a reminder to forward 2 additional copies of the efficacy analysis for Vap-02 for Submission.
58	September 17, 2004	E-Mail	Philip Schneider Genzyme	Bernhard Eggimann H3 Pharma	Notification from Genzyme – outcome of inspection	Summary of FDA's prior approval inspection on vapreotide acetate, September 15-17, 2004
59	September 22, 2004	E-Mail	J. Kay Noel J. Kay Noel & Associates	Diane Moore FDA	Submission of efficacy analysis VAP-02 (June/25)	Update on the status of outstanding information requests regarding NDA 21-761.
60	September 22, 2004	E-Mail	J. Kay Noel J. Kay Noel & Associates	Diane Moore FDA	Submission of dataset (September/13) and status update	NDA-21-761 response to request for information, Module 5, Vol 3.1 to 3.3

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
61	September 28, 2004	Fax	Diane Moore FDA	J. Kay Noel J. Kay Noel & Associates	Additional clinical questions	RE: DEB-96-VAP-14
62	October 7, 2004	E-Mail	J. Kay Noel J. Kay Noel & Associates	Diane Moore FDA	Responses to Sept 15 & 28 requests	Responses to the requests dated Sept 15 and 28, 2004 for clinical data for study DEB-96-VAP-14 and DEB-01-VAP-07
63	October 8, 2004	E-Mail	J. Kay Noel J. Kay Noel & Associates	Diane Moore FDA	Response to CMC questions (August 10)	Responses to request for CMC information dated 26 July, 2004. No outstanding information requests for NDA 21-761.
64	October 15, 2004	Fax	Diane Moore FDA	J. Kay Noel J. Kay Noel & Associates	Request for stats data set	Request for statistics related to DEB-01-VAP-07 and DEB-97-VAP-02
65	October 18, 2004	Fax	Diane Moore FDA	J. Kay Noel J. Kay Noel & Associates	Request for stats data set	Request for statistics related to DEB-96-VAP-14, DEB-01-VAP-07 and DEB-97-VAP-02
66	October 22, 2004	Letter	Paul Hofmann USAN Council, American Medical Association	Laura King H3 Pharma	Acknowledging H3's submission of October 10, 2004 of vapreotide Acetate for the treatment of acute variceal bleeding related to portal hypertension	File Number RR-39 assigned. Also receipt for cheque in amount of $USD 5,000.00

Number	Date of Communications	Type	From (Name, Organization)	To	Topic	Description
67	October 29, 2004	Fax	Diane Moore FDA	J. Kay Noel J. Kay Noel & Associates	Minutes of telecom of October 27, 2004	Minutes of October 27, 2004 teleconference
68	November 1, 2004	Letter	J. Kay Noel J. Kay Noel & Associates	Diane Moore FDA	Submission of datasets October 15 & 18, 2004	Responses to requests for statistical information dated Oct 15 & 18, 2004
69	December 1, 2004	Fax	Liang Zhou FDA	J. Kay Noel J. Kay Noel & Associates	CMC Discipline Review Letter	Letter advising that Chemistry, manufacturing and Controls section of submission is complete with deficiencies
70	December 21, 2004	Fax	Diane Moore FDA	J. Kay Noel J. Kay Noel & Associates	Approvable Letter	RE: NDA (NDA-21-761) dated February 27, 2004 for Sanvar (Vapreotide) Injection, 0.6 mg. Advising that application is approvable as amended pending certain additional information.
71	December 22, 2004	Letter	J. Kay Noel J. Kay Noel & Associates	Tanya D. Clayton FDA	Response advising of intent to file amendment	Response to FDA Action Letter dated December 21, 2004 advising that H3 Pharma intends to file an amendment that addresses all the deficiencies identified in the FDA letter
72	11 January 2005	Fax	J. Kay Noel J. Kay Noel & Associates	Laura King H3 Pharma	Minutes of December 17 Teleconference	Minutes of Dec 17 Teleconference between H3, FDA, J. Kay Noel, Debio, Expert

EXHIBIT 9

Power of Attorney

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No. : 4,650,787
Patentee : Schally et al.
Issue Date : March 17, 1987
Serial No. : 727,105
Filed : April 25, 1985
Title : BIOLOGICALLY ACTIVE OCTAPEPTIDES

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

POWER OF ATTORNEY

Pursuant to 37 C.F.R. § 1.730, H3 Pharma, Inc. ("H3 Pharma"), hereby appoints Scott B. Markow, Reg. No. 46,899 and Anita L. Meiklejohn, Ph.D., Reg. No. 35,283, of Fish & Richardson P.C., as its attorneys to prosecute the application for patent term extension of U.S. Patent No. 4,650,787 and to transact all business in the Patent and Trademark Office connected therewith with full powers of substitution and revocation.

The undersigned, an authorized representative of H3 Pharma, verifies that H3 Pharma is the exclusive agent of Debiopharm S.A. and Debio Recherche Pharmaceutique S.A. (collectively "Debio") for the purposes of pursuing a patent term extension for the '787 patent, by virtue of the Authorization for H3 Pharma to Apply for Extension of Patent Term ("Authorization"). In the Authorization, Debio certifies that: (i) The Tulane Educational Fund ("Tulane") is the assignee of 100% of the right, title and interest in the '787 patent; (ii) Debio has authority to seek a patent term extension for the '787 patent by virtue of Tulane having granted Debio an exclusive license to all of the rights of the '787 patent; and (iii). Debio has granted H3 Pharma a sublicense to the '787 patent, including the rights to develop and commercialize vapreotide.

Please direct all communications regarding the application to the attorney at the address and telephone numbers indicated below.

Scott B. Markow
FISH & RICHARDSON P.C.
1425 K Street, N.W.
11th Floor
Washington, DC 20005-3500
Telephone: (202) 783-5070
Facsimile: (202) 783-2331

Signature:  Date: March 24, 2005

Name: Loïc Maurel, MD

Title: President & CEO

H3 Pharma, Inc.